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(54) Title: VACCINE

(57) Abstract: The present invention provides a vaccine or immunogenic composition comprising: an immunogenic SARS coronavirus S (spike) polypeptide, or a fragment or variant thereof; and an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.

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VACCINE

Field of the invention

The present invention relates to vaccines against severe acute respiratory syndrome coronavirus (SARS-CoV) infection, and their use in the prevention of SARS. The invention also relates to methods of producing such vaccines.

Background to the invention

Coronavirus has a positive-sense, non-segmented, single-stranded RNA genome, which encodes at least 18 viral proteins including the structural proteins E, M, N and S. The S (spike) protein, a major antigen of coronavirus, is a membrane glycoprotein (200-220 kDa) which exists in the form of spikes emerging from the surface of the viral envelope. It is responsible for the attachment of the virus to the receptors of the host cell and for inducing the fusion of the viral envelope with the cell membrane. The S protein has two domains: S1, which is believed to be involved in receptor binding; and S2, believed to mediate membrane fusion between the virus and target cell (Holmes and Lai, 1996). The S protein can form non-covalently linked homotrimers (oligomers), which may mediate receptor binding and virus infectivity.

In March 2003, a new coronavirus (SARS-CoV or SARS virus) was isolated, in association with cases of severe acute respiratory syndrome (SARS). Genomic sequences of this new coronavirus have been obtained, including those of the Urbani isolate (Genbank accession No. AY274119.3 and A. MARRA et al., Science, May 1, 2003, 300, 1399-1404) and the Toronto isolate (Tor2, Genbank accession No. AY278741 and A. ROTA et al., Science, 2003, 300, 1394-1399).

Another strain of SARS-associated coronavirus has also been identified, which is distinguishable from the Tor2 and Urbani isolates. This coronavirus strain is derived from the sample collected from the bronchoaleveolar washings from a patient suffering from SARS, recorded under the No. 031589 and collected at the Hanoi (Vietnam) French hospital (WO 2005/056781 and WO 2005/056584).

Summary of the invention

The present invention provides a vaccine composition comprising an immunogenic SARS coronavirus S (spike) polypeptide, or a fragment or variant thereof, and an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.

The invention also provides a method of producing a vaccine composition of the invention, the method comprising combining an immunogenic S polypeptide, or a fragment or variant thereof, with an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.

The invention further provides:

- a vaccine composition of the invention for use as a medicament;
- a vaccine composition of the invention for the prevention or treatment of severe acute respiratory syndrome or other SARS-CoV-related disease;
- use of a vaccine composition of the invention for the manufacture of a medicament for the prevention or treatment of severe acute respiratory syndrome or other SARS-CoV-related disease;
- a method of preventing or treating severe acute respiratory syndrome or other SARS-CoV-related disease, which method comprises administering an effective amount of a vaccine composition of the invention to an individual in need thereof; and
- an immunogenic composition comprising:
  - (a) an immunogenic SARS coronavirus S (spike) polypeptide, or a fragment or variant thereof; and
  - (b) an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.

Brief description of the drawings

Figure 1 shows the effect of adjuvants on the humoral response induced by the Ssol polypeptide. Young adult BALB/c mice (8 per group) were immunised, at three week intervals, by two intramuscular injections of 2μg of Ssol protein without adjuvant (no adj.) or associated with 50μg of Alum or with 50μL of the 3D-MPL/QS21/liposome adjuvant (GSK1 adj.). Two control groups were immunised with each of the adjuvants alone. The sera were collected three weeks after each injection (IS1 and IS2, respectively), and the specific antibody response to the SARS-

CoV native antigens measured by anti-SARS ELISA as described in Callendret et al. (Virology, 2007, 363 : 288–302). The titers from each mouse are represented by black dots, and the averages by horizontal bars. The detection limit of the experiment is represented by a dotted line.

Figure 2 shows the effect of adjuvants on the neutralising humoral response induced by the Ssol polypeptide. Young adult BALB/c mice (8 per group) were immunised as described above. The neutralising antibody titers of sera collected three weeks after the last injection were measured as described in Callendret et al. (Virology, 2007, 363 : 288–302). The titers from each mouse are represented by dots, and the averages by horizontal bars. The detection limit of the experiment is represented by a dotted line.

Figure 3 shows modulation of the immune response type induced by the Ssol protein in the BALB/c mouse by using adjuvants. The specific IgG1 and IgG2a isotype titers to the SARS-CoV native antigens were measured on the mice sera collected 3 weeks after the immunisation. The titers measured for each mouse are shown as dots. For the control groups, the titers were measured on the mix of sera from each group, and shown by a diamond shape. The detection limit of the experiment is shown by a dotted line.

Figure 4 shows the effect of adjuvants on the humoral response induced by the Ssol polypeptide in Syrian Golden hamsters. The sera were collected three weeks after each injection (IS1 and IS2, respectively) and three months after the second injection (IS2bis), and the specific antibody response to the SARS-CoV native antigens measured by anti-SARS ELISA as in Figure 1. The titers from each hamster are represented by black dots and the averages by horizontal bars.

Figure 5 shows the effect of adjuvants on the neutralising humoral response induced by the Ssol polypeptide in Syrian Golden hamsters. The neutralising antibody titers of sera collected three months after the last injection were measured as described in Figure 2. The titers from each hamster are represented by dots and the averages by horizontal bars.

Figures 6 and 7 show the effect of adjuvants on the protective immune response induced by the Ssol polypeptide in Syrian Golden hamsters. Three months after the second injection, hamsters were challenged intranasally with 10^5 PFU of SARS-CoV. Four days after inoculation, hamsters were euthanized. Lungs and upper respiratory tract (URT, i.e. pharynx plus trachea) homogenates were prepared and

titrated for infectious SARS-CoV by plaque assay on Vero cells, as described in Callendret et al. (Virology, 2007, 363 : 288–302). Values for each individual hamster are represented with black circles for lung (figure 6) and URT (figure 7), and means with horizontal bars. The detection limits of the assays are indicated by a dotted line.

Figure 8 shows the results of histopathological analysis of the lungs of challenged hamsters previously immunized with 0.2 µg of Ssol protein. The scores of pulmonary inflammation and lesions (HE) and the scores of viral antigen loads (IHC) are shown on a 1-10 scale.

Figure 9 shows SARS-CoV specific IgG antibody titers determined by indirect ELISA from serum obtained on day 14 post-immunization from BALB/c mice immunized with different doses of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/ liposome (GSK1 adj.).

Figure 10 shows SARS-CoV isotype antibody titers determined by indirect ELISA from serum obtained on day 14 post-immunization from BALB/c mice immunized with 2 µg of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 11 shows SARS-CoV neutralizing antibody titers determined from serum obtained on day 14 post-immunization from BALB/c mice immunized with 0.2µg of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 12 shows CD4+ T cell response in PBMC obtained on day 7 post-immunization from BALB/c mice immunized with different doses of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 13 shows CD4+ T cell response in spleen obtained on day 14 post-immunization from BALB/c mice immunized with different doses of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 14 shows cytokine secretion (IL-5, IL-13 and IFN-γ) from spleen cells obtained on day 14 post-immunization from BALB/c mice immunized with different doses of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 15 shows SARS-CoV specific IgG antibody titers determined by indirect ELISA from serum obtained on day 14 post-immunization from C57BL/6

mice immunized with different doses of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/ liposome (GSK1 adj.).

Figure 16 shows SARS-CoV isotype antibody titers determined by indirect ELISA from serum obtained on day 14 post-immunization from C57BL/6 mice immunized with 2 μg of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 17 shows SARS-CoV neutralizing antibody titers determined from serum obtained on day 14 post-immunization from C57BL/6 mice immunized with 0.2μg of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 18 shows CD4+ T cell response in PBMC obtained on day 7 post-immunization from C57Bl/6 mice immunized with different doses of Ssol, alone or adjuvanted with Alum or 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 19 shows CD4+ T cell response in spleen cells obtained on day 14 post-immunization from C57Bl/6 mice immunized with different doses of Ssol adjuvanted with 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 20 shows cytokine secretion (IL-5, IL-13 and IFN-γ) from spleen cells obtained on day 14 post-immunization from C57Bl/6 mice immunized with different doses of Ssol adjuvanted with 3D-MPL/QS21/liposome (GSK1 adj.).

Figure 21 shows the effect of adjuvants on the neutralising humoral response induced by 2 μg of the Ssol polypeptide in Syrian Golden hamsters. The neutralising antibody titers of sera collected eight months after the last injection were measured as described in Figure 2. The titers from each hamster are represented by dots and the averages by horizontal bars.

Figures 22 and 23 show the effect of adjuvants on the protective immune response induced by 2 μg of the Ssol polypeptide in Syrian Golden hamsters. Eight months after the second injection, hamsters were challenged intranasally with 10^5 PFU of SARS-CoV. Four days after inoculation, hamsters were euthanized. Lungs and upper respiratory tract (URT, i.e. pharynx plus trachea) homogenates were prepared and titrated for infectious SARS-CoV by plaque assay on Vero cells, as described in Callendret et al. (Virology, 2007, 363 : 288–302). Values for each individual hamster are represented with black circles for lung (figure S2) and URT

(figure S3), and means with horizontal bars. The detection limits of the assays are indicated by a dotted line.

Figure 24 shows the results of histopathological analysis of the lungs of challenged hamsters previously immunized with 2 μg of Ssol protein. The scores of pulmonary inflammation and lesions (HE) and the scores of viral antigen loads (IHC) are shown on a 1-10 scale.

Detailed description

The present invention provides a vaccine composition which is useful in the prevention or treatment of severe acute respiratory syndrome (SARS) or other SARS-CoV-related disease. The term "vaccine", as used in the present invention, refers to a composition that comprises an immunogenic component capable of provoking an immune response in an individual, such as a human, optionally when suitably formulated with an adjuvant. Accordingly, in one embodiment the invention provides an immunogenic composition comprising an immunogenic SARS coronavirus S (spike) polypeptide, or a fragment or variant thereof, and an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.

The vaccine composition of the present invention comprises immunogenic SARS coronavirus S (spike) polypeptides, including fragments and variants thereof. The immunogenic S polypeptides may comprise any portion of an S protein that has an epitope capable of eliciting a protective immune response, for example an epitope capable of eliciting production of a neutralizing antibody and/or stimulating a cell-mediated immune response, against a SARS-CoV infection.

An exemplary SARS-CoV S protein has 1,255 amino acids (see for example SEQ ID NO:1), with a 13 amino acid signal sequence, the S1 domain at amino acids 12-672, and the S2 domain at amino acids 673-1192. The protein consists of a signal peptide (amino acids 1-13), an extracellular domain (amino acids 14-1195), a transmembrane domain (amino acids 1196-1218) and an intracellular domain (amino acids 1219-1255). The S protein sequence may be derived from any SARS-CoV strain, including those known to have caused SARS in human populations, for example the Tor2, Urbani or No. 031589 strains, or from any other strain of SARS-CoV, for example a strain that is circulating in an animal population, such as civets or bats, that has not yet entered the human population.

In one embodiment, the immunogenic S polypeptide is a portion or fragment of the full-length S protein. As described herein, an immunogenic S polypeptide includes a fragment of S protein or a S protein variant (which may be a variant of a full-length S protein or S fragment as described herein) that has at least one epitope contained within the full-length S protein or wildtype S protein, respectively, that elicits a protective immune response against SARS coronavirus.

In one embodiment, the immunogenic S polypeptide may consist of or comprise the entire extracellular domain (ectodomain) of the S protein, for example amino acids 1 to 1193. Hence, the immunogenic S polypeptide may consist of the S glycoprotein with its intracytoplasmic and transmembrane domains deleted. Optionally, the signal peptide (amino acids 1 to 13) may be deleted. In one embodiment, the immunogenic S polypeptide consists of the extracellular domain of the S protein extended to its C-terminus by a Serine-Glycine linker (SG) and octapeptide Flag (DYKDDDDK). In particular, the immunogenic S polypeptide may consist of or comprise amino acids 14 to 1193 of the SARS-CoV S protein fused at the C-terminal to the sequence SGDYKDDDDK. In a further embodiment, the S polypeptide may consist of or comprise the sequence of SEQ ID NO: 2.

An S protein fragment that comprises an epitope that stimulates, induces, or elicits an immune response may comprise a sequence of consecutive amino acids ranging from any number of amino acids between 8 amino acids and 150 amino acids (*e.g.*, 8, 10, 12, 15, 18, 20, 25, 30, 35, 40, 50, etc. amino acids) of SEQ ID NO: 1.

In other embodiments, a coronavirus S polypeptide variant has at least 50% to 100% amino acid identity (that is, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity) to the amino acid sequence of the full length S protein as set forth in SEQ ID NO: 1. Such S polypeptide variants and fragments may retain at least one S protein-specific biological activity or function, such as: (1) the capability to elicit a protective immune response, for example, a neutralizing response and/or a cell-mediated immune response against SARS-CoV; (2) the capability to mediate viral infection via receptor binding; and (3) the capability to mediate membrane fusion between a virion and the host cell.

An S polypeptide may contain conservative amino acid substitutions. Examples of conservative substitutions include substituting one aliphatic amino acid for another, such as Ile, Val, Leu, or Ala, or substituting one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn. A similar amino acid or

a conservative amino acid substitution is also one in which an amino acid residue is replaced with an amino acid residue having a similar side chain, which include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine); acidic side chains (*e.g.*, aspartic acid, glutamic acid); uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, histidine); nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); beta-branched side chains (*e.g.*, threonine, valine, isoleucine), and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan). Proline, which is considered more difficult to classify, shares properties with amino acids that have aliphatic side chains (*e.g.,* Leu, Val, Ile, and Ala). In certain circumstances, substitution of glutamine for glutamic acid or asparagine for aspartic acid may be considered a similar substitution in that glutamine and asparagine are amide derivatives of glutamic acid and aspartic acid, respectively.

Conservative and similar substitutions of amino acids in the coronavirus immunogen sequences disclosed herein may be readily prepared according to methods described herein and practiced in the art and which provide variants retaining similar physical properties and functional or biological activities, such as, for example, the capability to induce or elicit an immune response, which may include a humoral response (that is, eliciting antibodies that bind to and have the same biological activity as an antibody that specifically binds to the wildtype (or nonvariant) immunogen and/or that binds to antibodies that specifically bind to the wildtype or nonvariant immunogen). An S protein immunogen variant thereof may, for example, retain the capability to bind to cellular receptors and to mediate infectivity.

As used herein, "percent identity" or "% identity" is the percentage value returned by comparing the whole of the subject polypeptide, peptide, or variant thereof sequence to a test sequence using a computer implemented algorithm, typically with default parameters. The variant immunogens described herein could be made to include one or more of a variety of mutations, such as point mutations, frameshift mutations, missense mutations, additions, deletions, and the like, or the variants can be a result of modifications, such as by certain chemical substituents, including glycosylation and alkylation.

As described herein, S protein immunogens, fragments, and variants thereof described herein contain an epitope that elicits or induces an immune response, for instance a protective immune response, which may be a humoral response and/or a

cell-mediated immune response. A protective immune response may be manifested by at least one of the following: preventing infection of a host by a coronavirus; modifying or limiting the infection; aiding, improving, enhancing, or stimulating recovery of the host from infection; and generating immunological memory that will prevent or limit a subsequent infection by a SARS coronavirus. A humoral response may include production of antibodies that neutralize infectivity, lyse the virus and/or infected cell, facilitate removal of the virus by host cells (for example, facilitate phagocytosis), and/or bind to and facilitate removal of viral antigenic material. A humoral response may also include a mucosal response, which comprises eliciting or inducing a specific mucosal IgA response.

Induction of an immune response in a subject or host (human or non-human animal) by a SARS-CoV S polypeptide, fragment, or variant described herein, may be determined and characterized by methods described herein and routinely practiced in the art. These methods include *in vivo* assays, such as animal immunization studies, for example, using a rabbit, mouse, ferret, civet cat, African green monkey, or rhesus macaque model, and any one of a number of *in vitro* assays, such as immunochemistry methods for detection and analysis of antibodies, including Western immunoblot analysis, ELISA, immunoprecipitation, radioimmunoassay, and the like, and combinations thereof.

Other methods and techniques that may be used to analyze and characterize an immune response include neutralization assays (such as a plaque reduction assay or an assay that measures cytopathic effect (CPE) or any other neutralization assay practiced by persons skilled in the art). These and other assays and methods known in the art can be used to identify and characterize S protein immunogens and variants thereof that have at least one epitope that elicits a protective humoral or cell-mediated immune response against SARS coronavirus. The statistical significance of the results obtained in the various assays may be calculated and understood according to methods routinely practiced by persons skilled in the relevant art.

The coronavirus S protein immunogens (full-length proteins, variants, or fragments thereof), as well as corresponding nucleic acids encoding such immunogens, are provided in an isolated form, and in certain embodiments, are purified to homogeneity. As used herein, the term "isolated" means that the nucleic acid or polypeptide is removed from its original or natural environment.

A SARS coronavirus S protein immunogen and fragments and variants thereof may be produced synthetically or recombinantly. A coronavirus protein fragment that contains an epitope that induces an immune response against coronavirus may be synthesized by standard chemical methods, including synthesis by automated procedure. Alternatively, the S protein immunogens may be produced recombinantly. For example, the S protein immunogen may be expressed from a polynucleotide that is operably linked to an expression control sequence, such as a promoter, in a nucleic acid expression construct. For example, the S protein immunogen may be encoded by the DNA sequence of SEQ ID NO: 3 or 4. The SARS coronavirus S polypeptides and fragments or variants thereof may be expressed in mammalian cells, yeast, bacteria, insect or other cells under the control of appropriate expression control sequences. Cell-free translation systems may also be employed to produce such coronavirus proteins using nucleic acids, including RNAs, and expression constructs. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are routinely used by persons skilled in the art and are described, for example, by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, NY, (1989) and Third Edition (2001), and may include plasmids, cosmids, shuttle vectors, viral vectors, and vectors comprising a chromosomal origin of replication as disclosed therein.

As will be appreciated by those of ordinary skill in the art, a nucleotide sequence encoding a coronavirus S polypeptide or variant thereof may differ from the sequences presented herein due to, for example, the degeneracy of the genetic code. A nucleotide sequence that encodes a coronavirus polypeptide variant includes a sequence that encodes a homologue or strain variant or other variant. Variants may result from natural polymorphisms or may be synthesized by recombinant methodology, for example to introduce an amino acid mutation, or chemical synthesis, and may differ from wild-type polypeptides by one or more amino acid substitutions, insertions, deletions, and the like.

Adjuvant TH1 and TH2 immune responses

An immune response may be broadly divided into two extreme categories, being a humoral or cell mediated immune response (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These

categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a range of immunoglobulin isotypes including in mice IgG1.

It can be considered that the driving forces behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute, and can take the form of a continuum between these two extremes. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF-γ cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6, IL-10 and tumour necrosis factor-β(TNF-β).

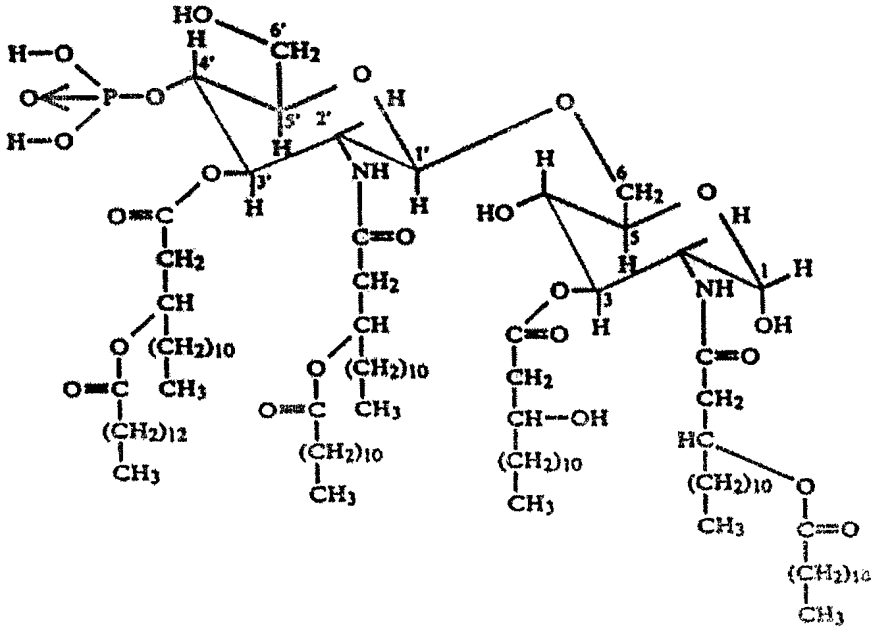
It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement (at least in mice) of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and induces antigen specific immunoglobulin responses associated with TH1-type isotype.

Lipopolysaccharide component

The composition according to the invention comprises an adjuvant which is a lipopolysaccharide. The lipopolysaccharide may be a non-toxic derivative of lipid A, such as monophosphoryl lipid A or more particularly 3-Deacylated monophoshoryl lipid A (3D–MPL).

It has long been known that enterobacterial lipopolysaccharide (LPS) is a potent stimulator of the immune system, although its use in adjuvants has been curtailed by its toxic effects. A non-toxic derivative of LPS, monophosphoryl lipid A (MPL), produced by removal of the core carbohydrate group and the phosphate from the reducing-end glucosamine, has been described by Ribi et al (1986, Immunology and Immunopharmacology of bacterial endotoxins, Plenum Publ. Corp., NY, p407-419) and has the following structure:



A further detoxified version of MPL results from the removal of the acyl chain from the 3-position of the disaccharide backbone, and is called 3-O-Deacylated monophosphoryl lipid A (3D-MPL). 3D-MPL is sold under the name MPL by

GlaxoSmithKline Biologicals N.A. and is referred to herein as MPL or 3D-MPL (see, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). It can be purified and prepared by the methods taught in GB 2122204B. Chemically it is a mixture of 3-deacylated monophosphoryl lipid A with 3, 4, 5 or 6 acylated chains.

Small particle 3D-MPL has a particle size such that it may be sterile-filtered through a 0.22μm filter, for example a particle size of less than 100nm in diameter. A method of manufacturing small particle 3D-MPL is disclosed in WO 94/21292. Aqueous formulations comprising monophosphoryl lipid A and a surfactant have been described in WO9843670.

Saponin component

The vaccine composition of the invention further comprises a saponin adjuvant component, optionally presented in the form of a liposome. A suitable saponin for use in the present invention is Quil A and its derivatives. Quil A is a saponin preparation isolated from the South American tree *Quillaja Saponaria Molina* and was first described by Dalsgaard *et al.* in 1974 ("Saponin adjuvants", Archiv. für die gesamte Virusforschung, Vol. 44, Springer Verlag, Berlin, p243-254) to have adjuvant activity. Purified fragments of Quil A have been isolated by HPLC which retain adjuvant activity without the toxicity associated with Quil A (EP 0 362 278), for example QS7 and QS21 (also known as QA7 and QA21). QS-21 is a natural saponin derived from the bark of *Quillaja saponaria* Molina, which induces CD8+ cytotoxic T cells (CTLs), Th1 cells and a predominant IgG2a antibody response.

In a suitable form of the present invention, the saponin adjuvant within the composition is a derivative of saponaria molina quil A, for example an immunologically active fraction of Quil A, such as QS-17 or QS-21, suitably QS-21. In one embodiment the compositions of the invention contain the immunologically active saponin fraction in substantially pure form, that is to say, the QS21 is at least 90% pure, for example at least 95% pure, or at least 98% pure.

In a specific embodiment, QS21 is provided in its less reactogenic composition where it is quenched with an exogenous sterol, such as cholesterol for example. Several particular forms of less reactogenic compositions wherein QS21 is quenched with an exogenous cholesterol exist. In a specific embodiment, the saponin/sterol is in the form of a liposome structure (WO 96/33739, Example 1).

Liposome formulation

The liposomes suitably contain a neutral lipid, for example phosphatidylcholine, which is suitably non-crystalline at room temperature, for example egg-yolk phosphatidylcholine, dioleoyl phosphatidylcholine (DOPC) or dilauryl phosphatidylcholine. The liposomes may also contain a charged lipid (sterol) which increases the stability of the lipsome-saponin structure for liposomes composed of saturated lipids. The ratio of saponin:sterol will typically be in the order of 1:100 to 1:1 (w/w), suitably between 1:10 to 1:1 (w/w), and usually from 1:5 to 1:1 (w/w). Suitably excess sterol is present, the ratio of saponin:sterol being at least 1:2 (w/w). In one embodiment, the ratio of saponin:sterol is 1:5 (w/w).

Suitable sterols include β-sitosterol, stigmasterol, ergosterol, ergocalciferol and cholesterol. In one particular embodiment, the vaccine composition comprises cholesterol as sterol. These sterols are well known in the art, for example cholesterol is disclosed in the Merck Index, 11th Edn., page 341, as a naturally occurring sterol found in animal fat.

Adjuvanted compositions of the invention comprising QS21 and a sterol, cholesterol in particular, show a decreased reactogenicity when compared to compositions in which the sterol is absent, while the adjuvant effect is maintained. Reactogenicity studies may be assessed according to the methods disclosed in WO 96/33739. The sterol according to the invention is taken to mean an exogenous sterol, for example a sterol which is not endogenous to the organism from which the antigenic preparation is taken but is added to the antigen preparation or subsequently at the moment of formulation. Typically, the sterol may be added during subsequent formulation of the antigen preparation with the saponin adjuvant, by using, for example, the saponin in its form quenched with the sterol. Suitably the exogenous sterol is associated to the saponin adjuvant as described in WO 96/33739.

The liposomes may be initially prepared without MPL (as described in WO 96/33739), and MPL is then added. In this aspect of the invention, the MPL is therefore not contained within the vesicle membrane (known as MPL out). Compositions where the MPL is contained within the vesicle membrane (known as MPL in) also form an aspect of the invention. The antigen may be contained within the vesicle membrane or may be contained outside the vesicle membrane.

In a specific embodiment of the invention, the lipopolysaccharide is 3D-MPL and the immunologically active saponin is QS21. In a further embodiment of the invention, the adjuvant consists essentially of 3D-MPL and QS21 in a liposomal formulation comprising cholesterol. The 3D-MPL and QS21 are typically present in a ratio of about 1:1. In one specific embodiment, the vaccine composition comprises about 50μg of QS21, about 50μg of 3D-MPL and about 25μl of liposomes per human dose. In another specific embodiment, the vaccine composition comprises about 25μg of QS21, about 25μg of 3D-MPL and about 12.5μl of liposomes per human dose.

Vaccine formulation and administration

The amount of the protein of the present invention present in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccines. Such amount will vary depending upon which specific immunogen is employed and the type and amount of adjuvant used. An optimal amount for a particular vaccine may be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Generally, it is expected that each dose will comprise 1-1000μg of protein, for example 1-200μg, or 10-100μg. A typical dose will contain 10-50μg, for example 15-25μg, suitably about 20μg of protein. Alternatively, a "dose-sparing" approach may be used, for example in a pandemic situation. This is based on the finding that it is possible to provide the same protective effect using lower doses of antigen, due to the presence of an effective adjuvant. Accordingly, each human dose may contain a significantly lower quantity of protein, for example from 0.1 to 10μg, or 0.5 to 5μg, or 1 to 3μg, suitably 2μg protein per dose. By the term "human dose" is meant a dose which is in a volume suitable for human use. Generally this is between 0.3 and 1.5 ml. In one embodiment, a human dose is 0.5 ml.

Following an initial vaccination, subjects typically receive a boost after a 2 to 4 week interval, for example a 3 week interval, optionally followed by repeated boosts for as long as a risk of infection exists. In a specific embodiment of the invention, a single-dose vaccination schedule is provided, whereby one dose of S protein in combination with adjuvant is sufficient to provide protection against the SARS CoV, without the need for any boost after the initial vaccination.

The vaccines of the invention may be provided by any of a variety of routes such as oral, topical, subcutaneous, mucosal (typically intravaginal), intraveneous, intramuscular, intranasal, sublingual, intradermal and via suppository.

Immunisation can be prophylactic or therapeutic. The invention described herein is primarily but not exclusively concerned with prophylactic vaccination against SARS.

Appropriate pharmaceutically acceptable carriers or excipients for use in the invention are well known in the art and include for example water or buffers. Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press New York, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877.

The vaccines and immunogenic compositions of the invention comprise certain components as laid out above. In a further aspect of the invention the vaccine or immunogenic composition consists essentially of, or consists of, said components.

The present invention is now described with respect to the following examples which serve to illustrate the invention.

Example 1

Expression of a soluble form of the SARS CoV-S protein

In order to purify the ectodomain of the S protein in mammalian cells, a gene was constructed enabling the expression of a spike glycoprotein with its intracytoplasmic and transmembrane domains deleted. This polypeptide, called Ssol, comprises the entire extracellular domain of the S protein (amino acids 1 - 1193) extended to its C-terminus by a Serine-Glycine linker and octapeptide Flag. Since the membrane anchoring domain is deleted, the Ssol polypeptide is secreted into the culture media.

Constitutive expression of the Ssol polypeptide

TRIP lentiviral vectors were used to establish cell lines expressing the Ssol protein in a stable and constitutive way. These vectors are produced by the co-

transfection of a pTRIP plasmid vector, a p8.7 packaging plasmid and a pHCMV-VSV-G plasmid (Yee et al., 1994; Zennou et al., 2000; Zufferey et al.,1997).

To construct the TRIP vectors for expression of the Ssol protein, an expression cassette composed of: the CMVi/e promoter, the chimeric intron from pCI plasmid, the Ssol ORF and of one of the two viral export elements CTE or WPRE was transferred into a plasmid pTRIP-EF1 -EGFP instead of the EF1 promoter and of the GFP ORF. The plasmids thus produced, called pTRIP-Ssol-CTE and pTRIP-Ssol-WPRE were used to produce TRIP-Ssol-CTE and TRIP-Ssol-WPRE lentiviral vector stocks respectively. These vectors were used to transduce the FRhK-4 cells according to a series of 5 consecutive transduction cycles spaced over 24 hours. The transduced cells were cloned by limiting dilution, and the cell clones obtained selected depending on their marked secretion of the polypeptide Ssol polypeptide. To do this, a fixed number of cells from various clones were seeded in 35mm culture dishes, and the presence of the Ssol protein in the supernatant analysed by western blot 72 hours later.

A protein of the expected size (~180 kDa) was detected in the supernatants from all the clones, confirming the efficiency of the transduction protocol. However, the expression levels varied from one clone to the other, independently of the TRIPvector used to produce them. The FRhK-4-Ssol-CTE#3 cell clone enabled the highest concentrations of the Ssol protein to be obtained in the supernatants collected after 72 hours of culture. This clone was submitted to a second series of 5 transduction cycles and the selection process repeated to obtain second generation clones. The most productive second generation clone (FRhK-4-Ssol-CTE#30) was amplified and used to produce greater quantities of supernatant. Subsequently, using a capture ELISA test, using a range of purified Ssol as protein marker, it was possible to estimate that the Ssol protein was secreted into the supernatant of the FRhK-4-Ssol-CTE#30 clone at concentrations varying from 5 - 10 μg/ml. The optimum conditions for producing the Ssol protein were determined experimentally by acting on the cell density parameters, serum concentration, culture temperature and secretion duration.

Production and purification of the recombinant Ssol protein

For large-scale production of Ssol protein, lots of $1.5 - 2.10^8$ sub-confluent cells of the FRhK-4-Ssol-CTE#30 clone were incubated at 35°C for 4 days in 1 litre of DMEM-based culture media containing 0.5% of foetal calf serum. The supernatant

containing the secreted Ssol protein was concentrated on an ultrafiltration unit and purified by affinity chromatography on an anti-FLAG antibody column. The material fixed to the column was eluted under non denaturing conditions by competition with the Flag peptide, and then separated by gel filtration to eliminate the Flag peptide and the low molecular weight contaminants.

The purified material was analysed by SDS-PAGE and silver nitrate staining. An intense, diffuse band, characteristic of glycoproteins was displayed with the expected size for the Ssol polypeptide (180-200kDa). Analysis by Western blotting after SDS-PAGE using a specific rabbit polyclonal antibody of the S protein confirmed that the purified protein clearly corresponds to the ectodomain of the S protein. The degree of purity of the purified protein was estimated after SDS-PAGE and staining with ruby SYPRO. The quantification of fluorescence signals indicated that more than 90% of proteins eluted from the gel filtration were from the Ssol protein. The purified Ssol protein was next quantified with the help of a kit using the Bi-cinchoninic acid assay (BCA). After analysis of 3 independent productions, it was possible to obtain from 1.3 – 2.5 mg of Ssol protein per litre of culture supernatant. The overall purification yield, including all the stages (concentration, affinity purification and gel filtration) varies from 26 - 53%. The purified Ssol protein was then further characterised by N-terminal sequencing, mass spectrography and analytical ultra-centrifuging. From this it was determined that the purified Ssol protein is a soluble monomer of 182 kDa corresponding to the entire ectodomain of the S protein, but missing the signal peptide (amino acids 1 - 13).

Preparation of a 3D-MPL/QS21/liposome adjuvant

To prepare concentrated liposomes, a mixture of 40g dioleoyl phosphocholine (DOPC), 10g of cholesterol and 3g of 3D-MPL was solubilised in ethanol and then dried down under vacuum to obtain a lipidic film. The lipidic film was flushed under argon, stored at -20°C for several days, and then placed at room temperature for 1 hour. Phosphate buffered saline (50mM phosphate, 100mM NaCl, pH 6.1) was added to give a final DOPC concentration of 40 mg/ml, a cholesterol concentration of 10 mg/ml and a final 3D-MPL concentration of 2 mg/ml. The vessel was agitated until all the lipid was in suspension. This suspension was then homogenised until the liposome size was reduced to about 100 nm, and then sterile filtered through a 0.2 μm filter.

A two-fold concentrated form of the 3D-MPL/QS21/liposome adjuvant was prepared by mixing concentrated liposomes with QS21 in phosphate buffered saline (50mM phosphate, 100mM NaCl, pH 6.1). This mixture was then diluted to reach a final concentration of 200 µg/ml of 3D-MPL and 200 µg/ml of QS21.

The formulations were prepared extemporaneously according the following sequence: phosphate buffered saline + Ssol antigen (quantities were added in order to reach final concentrations of 40µg/ml or 4µg/ml or 0.4µg/ml), 5 min mixing on an orbital shaking table at room temperature, + 2-fold concentrated adjuvant, 5 min mixing on an orbital shaking table at room temperature. The injections occurred within two hours following the end of the formulation.

Testing adjuvanted vaccine in a mouse model

BALB/c young adult mice (8 per group) received two injections, at 3 week intervals, into muscular tissue, of 2µg of Ssol protein either without adjuvant, or with 50µg of Alum or 50µL of the 3D-MPL/QS21/liposome adjuvant (GSK1 adj.). These doses of adjuvants are traditionally used with small rodents and correspond to 1/10th of doses used in human medicine. Two groups of mice were associated with this research as controls, each being immunised with only one of the adjuvants. The mice sera were collected 3 weeks after each injection, and the specific humoral response of the SARS-CoV evaluated by anti-SARS ELISA, seroneutralisation and isotype analysis.

By ELISA (Figure 1), the titers in antibodies of sera from control groups constantly remained below the limit of detection (1.7 log10). After only one injection, the responses in antibodies induced by the protein with no adjuvant or with Alum adjuvant are weak (average titers of 1.9 ± 0.2 log10 and 2.1 ± 0.3 log10, respectively), confirming the results obtained previously. Contrariwise, the antibody titers induced by the protein with 3D-MPL/QS21/liposome adjuvant are very large from the first injection (average titer of 3.6 ± 0.2 log10). After two injections, a marked increase in titers in all the groups immunised with the protein is noted. The weakest response and the most heterogeneous one is observed when no adjuvant was used (average titer of 3.9 ± 0.5 log10). The adding of Alum to the immunogenic preparation enables the antibody response to be improved (average titer of 4.6 ± 0.2 log10; $p < 0.01$). Tallying with the results observed after the first injection, the 3D-MPL/QS21/liposome

adjuvant markedly improved the immunogenicity of the Ssol protein after two injections, and the antibody titers obtained (average titers of 5.2 ± 0.2 log10) are significantly higher than those induced by the protein with Alum adjuvant ($p < 10^{-4}$).

The quality of the humoral response by the various immunogens was studied on the sera collected 3 weeks after the second injection. The neutralising antibody titers (Figure 2) follow the hierarchy observed at the time of the analysis by ELISA. The weakest titers are obtained with the protein with no adjuvant (average titer of 2.3 ± 0.4 log10). The neutralising response is significantly improved by the addition of Alum (average titer of 3.1 ± 0.3 log10; $p < 0,001$). Likewise, the addition to the protein of 3D-MPL/QS21/liposome adjuvant enables very large neutralising antibody titers to be achieved (average titers of 3.6 ± 0.1 log10), and significantly threefold higher than those induced by the protein with Alum adjuvant ($p < 0.002$).

The specific IgG1 and IgG2a isotype titers to the SARS-CoV antigens were evaluated for each group by anti-SARS ELISA on the sera collected 3 weeks after the last injection (Figure 3). The immunisations with the protein with no adjuvant or with the protein with Alum adjuvant almost exclusively induce IgG1s. The addition to the Ssol protein of the 3D-MPL/QS21/liposome adjuvant enables high IgG2a titers to be induced (average titer 4.9 ± 0.2 log10) comparable to IgG1 titers (average titer of 4.8 ± 0.1 log10, average ratio IgG1 over IgG2a of 1). These results demonstrate that the immune responses induced by the protein with no adjuvant or with Alum are predominantly type 2. Contrariwise, the addition of the 3D-MPL/QS21/liposome adjuvant to the Ssol protein enables mixed immune response of TH1-type and TH2-type to be induced.

Example 2

Testing adjuvanted vaccine in a hamster model

Syrian golden hamsters (6 per group) were injected twice, at 3-week intervals, into muscular tissue, with 2µg or 0.2 µg of Ssol protein either with 50µg of Alum or 50µL of the 3D-MPL/QS21/liposome adjuvant (GSK1 adj.). These doses of adjuvants are traditionally used with small rodents and correspond to the 1/10th doses used in human medicine. Two groups of hamsters were associated with this experiment as controls, each being immunised with only one of the adjuvants. Another group of hamsters was injected with 2µg (S-equivalent) of purified and ß-propiolactone-

inactivated SARS-CoV virions (BPL-SCoV) with 50µg of Alum, which constitutes a potential vaccine against SARS. The hamster sera were collected 3 weeks after each injection (IS1 and IS2, respectively) and 3 months after the second injection (IS2bis), and the specific humoral response of the SARS-CoV evaluated by anti-SARS ELISA and seroneutralisation analysis.

By ELISA (Figure 4), the titers in antibodies of sera from control groups constantly remained below the limit of detection (1.7 log10). At the 2µg Ssol dose, the 3D-MPL/QS21/liposome adjuvant markedly improved the immunogenicity of the Ssol protein after one (IS1) and two (IS2) injections, and the antibody titers obtained (average titers of 4.3 ± 0.1 log10 and 4.9 ± 0.1 log10) are 0.6 log10 higher than those induced by the protein with Alum adjuvant ($p < 10^{-3}$).

After only one injection of 0.2µg of Ssol, the response observed when Alum adjuvant was used is weak and close to the limit of detection (average titers of 1.8 ± 0.2 log10). Contrariwise, the antibody titers induced by the protein with 3D-MPL/QS21/liposome adjuvant are high from the first injection (average titer of 3.5 ± 0.4 log10). After two injections, a marked increase in titers in both groups immunised with the protein is noted. The weakest response and the most heterogeneous one is observed when the Alum adjuvant was used (average titer of 2.6 ± 0.7 log10). The addition of 3D-MPL/QS21/liposome adjuvant to the immunogenic preparation enables the antibody response to be strongly improved (average titer of 4.7 ± 0.2 log10; $p < 10^{-4}$).

Remarkably, after the second injection, 0.2 µg and 2µg Ssol with 3D-MPL/QS21/liposome adjuvant achieved comparable high-titer responses in all immunized hamsters (4.7 ± 0.2 log10 versus 4.9 ± 0.1 log10 titer). This indicates that the use of 3D-MPL/QS21/liposome adjuvant could enable dose-sparing vaccine strategies against SARS.

The quality of the humoral response induced by 0.2 µg Ssol or 2µg (S-equivalent) inactivated virions was studied on the sera collected 3 months after the second injection. The neutralising antibody titers (Figure 5) follow the hierarchy observed at the time of the analysis by ELISA. The titers obtained with the protein with Alum remained below the limit of detection (1.3 log10). The neutralising response is strongly improved by the addition of 3D-MPL/QS21/liposome adjuvant (average titer of 2.6 ± 0.3 log10; $p < 10^{-6}$). This response was clearly similar to the

response induced by 2μg (S-equivalent) inactivated virions (average titer of 2.5 ± 0.2 log10).

Challenge infection of Ssol-immunized hamsters

At 3 months post-immunization, selected groups of hamsters were challenged by intranasal inoculation of 10^5 pfu of SARS-CoV and euthanized 4 days later in order to assess viral replication. Viral loads were evaluated in the lungs (Figure 6) and in the upper respiratory tract (URT), i.e. pharynx plus trachea (Figure 7) of each animal. We observed a robust and consistent replication of the virus in the lungs of each mock-immunized animal (7.8 ± 0.2 log10 pfu). In addition, viral replication was documented in the upper respiratory tract of mock-vaccinated hamsters (5.2 ± 0.5 log10 pfu). SARS-CoV loads remained detectable both in lungs (4.1 ± 1.4 log10 pfu) and URT (2.5 ± 0.4 log10 pfu) of animals immunized with 0.2μg Ssol with Alum. In sharp contrast, in spite of robust virus replication in this animal model, no infectious virus was detectable in any of the hamsters immunized with Ssol and 3D-MPL/QS21/liposome adjuvant (log10 pfu / organ < 2.1). These data provide evidence for a more than 10^2-fold reduction of SARS-CoV replication in the lungs of hamsters immunized with Ssol and 3D-MPL/QS21/liposome adjuvant compared to hamsters immunized with Ssol and Alum. This high level of protection achieved with Ssol and 3D-MPL/QS21/liposome adjuvant is comparable to that observed in hamsters immunized with inactivated virions and Alum.

Interestingly, a single injection of 2μg Ssol with 3D-MPL/QS21/liposome adjuvant induced similar high ELISA titers of anti-SARS antibodies (4.3 ± 0.1 log10 pfu) as 2 injections of 0.2μg Ssol with 3D-MPL/QS21/liposome adjuvant at the time of challenge (4.3 ± 0.3 log10 pfu) (figure 4). Given the fact that this latter vaccination schedule protects immunized hamsters against SARS challenge (Figure 6 and 7), it can be anticipated that a single injection of 2μg Ssol with 3D-MPL/QS21/liposome adjuvant will also induce a protective response. This indicates that the use of 3D-MPL/QS21/liposome adjuvant could enable single-dose vaccination strategies against SARS.

Histopathological analysis of the lungs of challenged hamsters

After challenge, the lungs of hamsters immunized with 0.2 µg of Ssol protein were subjected to histopathological examination using Hemalun-Eosin stain and immunohistochemistry analysis using anti-SARS-CoV polyclonal antibody. Figure 8 shows the scores of pulmonary inflammation and lesions (HE) and the scores of viral antigen loads (IHC) on a 1-10 scale. In mock vaccinated-animals, characteristic lesions of acute viral pneumonia were observed with diffuse lesions of exsudative alveolitis, diffuse condensation of the lung parenchyma and diffuse alveolar damage (score = 3.3 ± 0.6). Accordingly, viral antigens were detected within these foci of alveolitis and also in the epithelium of the trachea and the broncho-alveolar tree (score = 4.3 ± 0.6). Both lesion scores (2.6 ± 1.0) and viral antigen loads (3.3 ± 1.2) remained high in lungs of animals immunized with 0.2µg Ssol with Alum. In sharp contrast, in Ssol and 3D-MPL/QS21/liposome adjuvant-vaccinated hamsters, no specific lesion of alveolitis or pneumonia was detected in the lungs (score = 0.6 ± 0.2) and viral antigens were detected in few cells from the bronchial epithelium of a single hamster (score 0.1 ± 0.2). Extensive IHC screening of respiratory tract sections from the 5 other animals confirmed the absence of viral antigens in the upper respiratory tract (pharynx-trachea, data not shown) and lungs (Figure 8).

These results confirm that hamsters vaccinated with Ssol and 3D-MPL/QS21/liposome adjuvant were fully protected from SARS-CoV challenge, as indicated by an almost complete lack of detectable viral antigen in the upper and lower repiratory tracts, and the absence of pneumonitis.

Long term protection from challenge infection of Ssol-immunized hamsters

Long term protection was studied for the group of hamsters immunized twice with 2µg of Ssol. Eight months after the second injection, the neutralising antibody response was improved by the addition of 3D-MPL/QS21/liposome adjuvant (average titer of 2.5 ± 0.3 log10; p = 0.02) when compared to the addition of alum (Figure 21). This response was clearly similar to the response induced by 2µg (S-equivalent) inactivated virions (average titer of 2.6 ± 0.2 log10).

The hamsters were then challenged by intranasal inoculation of 10^5 pfu of SARS-CoV and euthanized 4 days later in order to assess viral replication. Viral loads were evaluated in the lungs (Figure 22) and in the upper respiratory tract (URT) (Figure 23) of each animal. Consistent with the results described above, a robust virus

replication was observed in both the lungs and URT of mock-vaccinated animals (7.8±0.2 log10 pfu and 5.4 ± 0.1 log10 pfu in the lungs and URT, respectively). In animals immunized with 2 μg Ssol with Alum, SARS-CoV loads were detectable in both the lungs and URT in 2 out of 5 animals and a high viral load (4.8 log10 pfu) was observed in the URT in one animal. In sharp contrast, no infectious virus was detectable in any of the hamsters immunized with Ssol and 3D-MPL/QS21/liposome adjuvant (log10 pfu / organ < 2.1). This high level of protection achieved with Ssol and 3D-MPL/QS21/liposome adjuvant is comparable to that observed in hamsters immunized with inactivated virions and Alum.

In addition, following challenge and euthanasia, the lungs of the hamsters were subjected to histopathological examination using Hemalun-Eosin stain (HE) and immunohistochemistry (IHC) analysis using anti-SARS-CoV polyclonal antibody (Figure 24). As described above, in mock vaccinated-animals, characteristic lesions of acute viral pneumonia were observed with diffuse lesions of exsudative alveolitis, diffuse condensation of the lung parenchyma and diffuse alveolar damage (score = 4.6 ± 0.2) and viral antigens were detected (score 5.3 ± 0.4). In the lungs of animals vaccinated with 2 μg of Ssol and 3D-MPL/QS21/liposome adjuvant, lesion scores were significantly reduced (0.9 ±0.6, $p<10^{-5}$) and viral antigen loads were undetectable whereas in animals vaccinated with 2 μg of Ssol with Alum, a more modest reduction of lesion scores was observed (score = 2.6 ± 0.8) and viral antigen remained detectable in 2 out of 5 animals (score = 0.5 ± 0.6).

Altogether, these data provide evidence for the potential for long term protection by the Ssol protein and 3D-MPL/QS21/liposome adjuvant.

Example 3

A) Humoral immune response to adjuvanted Ssol protein in BALB/c mice

Female BALB/c mice aged 6-8 weeks were obtained from Harlan Horst, The Netherlands. Mice (23 mice/group) were injected intramuscularly on days 0 and 21 with 2, 0.2 or 0.02 μg Ssol protein without adjuvant ("Plain"), adjuvanted with 50μg Alum or with the 3D-MPL/QS21/ liposome adjuvant. Three additional groups of mice

were included as controls, each being immunised with PBS, Alum or the 3D-MPL/QS21/ liposome adjuvant alone.

Preparation of non adjuvanted Ssol antigen

The formulations were prepared extemporaneously according to the following sequence: water for injection + Ssol antigen (quantities are added in order to reach final concentrations of 40µg/ml or 4µg/ml or 0.4µg/ml), 5 min mixing on an orbital shaking table at room temperature + NaCl 1500mM (in order to reach a final concentration of 150mM), 5 min mixing on an orbital shaking table at room temperature. The injections occurred within an hour following the end of the formulation.

Preparation of Alum-adjuvanted Ssol

The vaccine preparation was made according the following sequence: water for injection + aluminium hydroxide (quantities are added in order to reach a final concentration of 1000µg/ml) + Ssol antigen (in order to reach a final concentration of 40µg/ml, 4µg/ml or 0.4µg/ml), 30 min mixing on an orbital shaking table at room temperature + NaCl 1500mM (in order to reach a final concentration of 150mM), 5 min mixing at room temperature on an orbital shaking table. The vaccine was prepared six days before the first immunization in the first study and kept at 4°C until injection.

Preparation of 3D-MPL/QS21/ liposome-adjuvanted Ssol

A two fold concentrated form of 3D-MPL/QS21/ liposome adjuvant was prepared by mixing concentrated liposomes and QS21 in a PO_4 50mM/NaCl 100mM pH6.1 buffer. Concentrated liposomes were made of DOPC, cholesterol and 3D-MPL. The final concentration of MPL was 200µg/ml and the final concentration of QS21 was 200µg/ml. The formulations were prepared extemporaneously according the following sequence: water for injection + saline buffer (PO4 0.5M/NaCl 1M pH6.1) + Ssol antigen (quantities are added in order to reach final concentrations of 40µg/ml or 4µg/ml or 0.4µg/ml), 5 min mixing on an orbital shaking table at room temperature, + 2-fold concentrated adjuvant, 5 min mixing on an orbital shaking table at room temperature. The injections occurred within an hour following the end of the formulation.

Analysis of humoral response

The humoral response was evaluated on sera prepared from blood samples taken from individual mice (8 mice per group) at 14 days post-immunization (day 35 timepoint). Detection of the presence of anti-SARS-CoV specific antibodies and isotype analysis were performed by indirect ELISA using a lysate of VeroE6 cells infected by SARS-CoV as antigen or of non-infected VeroE6 cells as a negative control. Titers were calculated as the reciprocal of the dilution of serum giving an OD of 0.5 after revealing with polyclonal anti-mouse IgG(H+L) antibodies coupled to peroxydase (NA931V, Amersham) followed by addition of TMB and H2O2 (KPL). For the analysis of isotypes polyclonal sera specific for mouse IgG1 and IgG2a antibodies were used (Southern Biotech).

Anti-SARS-CoV antibodies.

A dose-dependent anti-SARS-CoV antibody response was observed in mice immunized with the Ssol protein either without adjuvant or in the presence of Alum or of 3D-MPL/QS21/liposome adjuvants (Figure 9). The antibody response was found to be significantly higher in mice immunized with Ssol in the presence of adjuvant as compared to mice immunized with non-adjuvanted Ssol. The response was significantly higher for mice immunized with the 3D-MPL/QS21/liposome-adjuvanted Ssol protein as compared to mice immunized with Alum-adjuvanted Ssol ($p<10^{-4}$); antibody titers induced with the lowest dose of Ssol (0.02 μg) in the presence of 3D-MPL/QS21/liposome -adjuvant were found superior to those induced with the highest dose of Ssol (2 μg) in the presence of alum ($p<0.01$).

Isotype analysis of anti-SARS-CoV antibodies.

An isotype analysis for IgG1 and IgG2a antibodies specific of SARS-CoV was performed by ELISA on sera prepared from the "Plain", Alum-adjuvanted and 3D-MPL/QS21/ liposome-adjuvanted groups immunized at a dose of 2 μg Ssol (8 mice per group). Results are shown in Figure 10.

In mice immunized either with the non-adjuvanted Ssol protein or with the Ssol protein adjuvanted with alum, the response was found to be strongly biased towards the IgG1 isotype whereas very low levels of IgG2a antibodies were detected. In mice immunized with the 3D-MPL/QS21/liposome-adjuvanted Ssol protein, high

titers of both IgG1 (4.9±0.2 log10 titers) and IgG2a (5.1±0.1 log10 titers) antibodies were reached.

Neutralizing antibodies

The presence of neutralizing antibodies was determined by a standard seroneutralization assay on FRhK-4 cells using 100 TCID50 of SARS-CoV per well. Serial two-fold dilutions of heat inactivated sera (56°C for 30 min) were used from dilution 1:20 on and tested in duplicate. Neutralizing titers were determined according to the method of Reed and Munsch (Am J Hyg 1938;27:493-97) as the reciprocal of the dilution that neutralizes virus infectivity in 50% of the wells (2 out of 4 wells).

Sera prepared 14 days post-immunization from individual mice immunized with 0.2 μg of Ssol either without or in the presence of alum or of 3D-MPL/QS21/liposome-adjuvant (8 mice/group) were analyzed for the presence of antibodies neutralizing SARS-CoV. Sera from mice immunized with an inactivated whole virus preparation at a dose equivalent to 0.5 μg S protein in the presence of 3D-MPL/QS21/liposome adjuvant were included for comparison. Results are shown in Figure 11.

In mice immunized with the 3D-MPL/QS21/liposome-adjuvanted Ssol protein, neutralizing antibody titers (3.5±0.3 log10 titers) were 0.7 log10 higher than in mice immunized with the alum-adjuvanted Ssol protein (2.8±0.3 log10 titers, $p<0.001$) whereas in mice immunized with the non-adjuvanted Ssol protein neutralizing titers remained undetectable for 6 out of 8 mice (<1.3 log10 titers). Noticeably, in the presence of 3D-MPL/QS21/liposome adjuvant, neutralizing antibody titers were comparable with 0.2 μg of Ssol protein as compared to 0.5 μg S-equivalent whole virus antigen.

B) Cellular immune response to adjuvanted Ssol protein in BALB/c mice

The cell-mediated immune responses of the "Plain", Alum-adjuvanted and 3D-MPL/QS21/ liposome-adjuvanted groups of BALB/c mice were investigated, as summarised in Table A below.

Table A

Read-out	Timepoint (days after 1st injection)	Sample type	Analysis method
CD4,CD8, IL-2, IFN-γ	D28	PBMC	Intracellular cytokine staining (ICS) (FACS analysis)
CD4,CD8, IL-2, IFN-γ	D35	Spleen	ICS (FACS analysis)
IL-5, IL-13 and IFN- γ	D35	Spleen	Cytometric Bead Array (CBA) (FACS analysis)

Cellular responses were measured on PBMC and spleens from 15 mice/group. PBMC were harvested 7 days post-immunization and spleens were harvested 14 days post-immunization. PBMC were tested on 5 pools of 3 mice and spleens were tested on 4 pools of 2 mice per group.

Intracellular cytokine staining (ICS)

After lysis of red blood cells with a lysis buffer (BD pharmingen), in vitro antigen stimulation of PBMC was carried out at a final concentration of 10^7 cells/ml (microplate 96 wells) with a concencentration of Ssol at 1 µg/ml final, and then incubated 2 hours at 37°C with the addition of anti-CD28 and anti-CD49d (1 µg/ml for both). Following the antigen restimulation step, cells were incubated overnight in presence of Brefeldin (1 µg/ml) at 37°C to inhibit cytokine secretion.

Spleens were collected from mice and pooled (4 pools of 2 mice/group) in medium RPMI+ Add. RPMI + Add-diluted PBL suspensions were adjusted to 10^7 cells/ml in RPMI 5% fetal calf serum. In vitro antigen stimulation of spleen cells was carried out with Ssol 1 µg/ml final and then incubated 2 hrs at 37°C with the addition of anti-CD28 and anti-CD49d (1 µg/ml for both). Following the antigen restimulation step, cells were incubated overnight in presence of Brefeldin (1 µg/ml) at 37°C to inhibit cytokine secretion.

After overnight at 4°c, cell staining was performed as follows: cell suspensions were washed, resuspended in 50 µl of PBS 1% FCS containing 2% Fc blocking reagent (1/50; 2.4G2). After 10 minutes incubation at 4°C, 50 µl of a mixture of anti-CD4-PE (1/50) and anti-CD8a perCp (1/50) was added and incubated 30 minutes at 4°C. After a washing in PBS 1% FCS, cells were permeabilized by resuspending in 200µl of Cytofix-Cytoperm (Kit BD) and incubated 20 min at 4°C.

Cells were then washed with Perm Wash (Kit BD) and resuspended with 50 µl of a anti- IFNγ-APC (1/50) + anti-IL-2 FITC (1/50) diluted in PermWash. After 2 hours incubation at 4°C, cells were washed with Perm Wash and resuspended in PBS 1% FCS + 1% paraformaldehyde. Sample analysis was performed by FACS. Live cells were gated (FSC/SSC) and acquisition was performed on ~ 20,000 events (lymphocytes CD4). The percentages of IFNγ + or IL2+ were calculated on CD4+ gated populations.

Dosage of cytokines in restimulation supernatant was also performed on PBMC from spleen 14 days after the immunization. Spleen was collected from mice and pooled (4 pools of 2 mice/group) in medium RPMI+ Add. PBMC suspensions were adjusted to 10^7 cells/ml in RPMI 5% fetal calf serum. In vitro antigen stimulation of PBMC was carried out with Ssol 1 µg/ml final and then incubated 72 hrs at 37°C. The supernatant was harvested and stored at -70°c until testing by CBA (Cyokine Bead Assay)-flex (BD Kit) for IFNγ, IL-5 and IL-13 detection.

CD4+ T cell responses in PBMC

At each antigen dose, significantly higher ($p<0.05$) CD4+ T cell responses were induced in mice immunized with 3D-MPL/QS21/liposome-adjuvanted Ssol protein compared to mice immunized with Alum-adjuvanted Ssol or the non-adjuvanted Ssol protein (Figure 12). Alum-adjuvanted Ssol or the non-adjuvanted antigen induced a similar level of CD4+ T cell responses as achieved by immunization with adjuvants alone or PBS. A trend for higher CD4+ T cell responses was observed after immunization of mice with 0.2 µg Ssol protein adjuvanted with 3D-MPL/QS21/liposome compared to mice immunized with 2 µg ($p = 0.04$, but difference < 2.5 fold) or 0.02 µg ($p=0.07$) of 3D-MPL/QS21/liposome-adjuvanted Ssol protein.

CD4+ T cell responses in spleen

At each antigen dose, higher CD4+ T cell responses were induced in mice immunized with 3D-MPL/QS21/liposome-adjuvanted Ssol protein compared to mice immunized with Alum-adjuvanted Ssol or the non-adjuvanted Ssol protein (Figure 13). Alum-adjuvanted Ssol or the non-adjuvanted antigen induced a similar level of CD4+ T cell responses as achieved by immunization with adjuvants alone or PBS. A trend for higher CD4+ T cell responses was observed after immunization of mice with 0.2 or 0.02 μg Ssol protein adjuvanted with 3D-MPL/QS21/liposome compared to mice immunized with 2 μg of 3D-MPL/QS21/liposome-adjuvanted Ssol protein.

Cytokine secretion from spleen cells

Bead populations with distinct fluorescence intensities were coated with capture antibodies specific for IFN-γ, IL5 and IL-13 proteins. Bead populations were mixed together to form the cytometric bead array (CBA) that was resolved in the FL3 channel of a BD FACS brand flow cytometer. The cytokine capture beads were mixed with the PE-conjugated detection antibodies and then incubated with recombinant standards or test samples to form sandwich complexes. Following acquisition of the sample data using the flow cytometer, the sample results were generated in graphical and tabular format.

Mouse cytokine standards were reconstituted and diluted by serial dilutions using the assay diluent. Mouse cytokine capture bead suspensions were pooled, mixed and transferred to each assay tube (50 μl/tube). Standard dilutions and test samples were added to the appropriate sample tubes (50 μl/tube) followed by 50 μl of PE detection reagent. All samples and standards were incubated for 2 hours at room temperature in the dark. After the incubation, all reaction tubes were washed with 1 ml of wash buffer, and centrifuged at 200 x g for 5 minutes. After decanting, standards and samples were resuspended in 300 μl of wash buffer. Standards and samples were read on a FACSCalibur flow cytometer with BD FACSComp software for setting up the cytometer and CellQuest_software for the analysis of the samples, followed by a subsequent analysis (calculation of sample concentrations with standard curve) using the BD CBA software.

Cytokine secretion from spleen cells.

Higher levels of IL-13 and IFN-γ secretion were induced in mice immunized with Alum-adjuvanted Ssol or 3D-MPL/QS21/liposome-adjuvanted Ssol protein compared to mice immunized with the non-adjuvanted Ssol protein (Figure 14). For both adjuvants (Alum and 3D-MPL/QS21/Liposome), this difference was statistically significant for both IL-13 and IFN-γ with a dose of 0.2 and 0.02 µg Ssol protein. Trend for higher levels of IL-5 were induced in mice immunized with Alum-adjuvanted Ssol compared to mice immunized with Ssol protein plain or adjuvanted with 3D-MPL/QS21/liposome confirming a trend for higher Th2-type profile with Alum. Nevertheless, a mixed Th1-type (IFN-γ) and Th2-type (IL-5 and IL-13) cytokines were induced by Alum and 3D-MPL/QS21/liposome adjuvants compared to the non-adjuvanted Ssol protein. No antigen dose response was observed in mice receiving different amounts of 3D-MPL/QS21/liposome-adjuvanted Ssol protein or the non-adjuvanted Ssol protein.

Example 4

A) Humoral immune responses to adjuvanted Ssol protein in C57Bl/6 mice

The same experimental protocol as described in Example 3 for BALB/c mice was carried out on female C57Bl/6 mice aged 6-8 weeks obtained from Harlan Horst, The Netherlands. For the analysis of isotypes polyclonal sera specific for mouse IgG1 and IgG2b antibodies were used (Southern Biotech).

Anti-SARS-CoV antibodies

A dose-dependent anti-SARS-CoV antibody response was observed in mice immunized with the Ssol protein either without adjuvant or in the presence of Alum or of 3D-MPL/QS21/liposome adjuvants (Figure 15). At each antigen dose, the antibody response was found to be significantly (0.5-2 log10) higher in mice immunized with Ssol in the presence of adjuvant as compared to mice immunized with non-adjuvanted Ssol. At the 2µg and 0.2µg antigen dose, the response was significantly (0.5-1.1log10) higher for mice immunized with the 3D-MPL/QS21/ liposome-adjuvanted Ssol protein ($p<0.01$) as compared to mice immunized with the Alum-adjuvanted Ssol protein. A trend for higher antibody response was observed after immunization of

mice with 0.02μg Ssol adjuvanted with 3D-MPL/QS21/ liposome compared to mice immunized with alum-adjuvanted (p=0.05, and difference = 0.5log10) or plain (p=0.03 Ssol, and difference = 0.6log10) Ssol protein.

Isotype analysis of anti-SARS-CoV antibodies

In mice immunized either with the non-adjuvanted Ssol protein or with the Ssol protein adjuvanted with alum, the response was found to be strongly biased towards the IgG1 isotype whereas no or very low levels of IgG2b antibodies were detected (Figure 16). In mice immunized with the 3D-MPL/QS21/liposome-adjuvanted Ssol protein, high titers of both IgG1 (4.5±0.3 log10 titers) and IgG2b (4.8±0.3 log10 titers) antibodies were reached.

Neutralizing antibodies

In mice immunized with the 3D-MPL/QS21/liposome-adjuvanted Ssol protein, neutralizing antibody titers (2.6±0.4 log10 titers) were significantly higher than in mice immunized with the alum-adjuvanted Ssol protein (1.8±0.4 log10 titers, $p<0.01$) whereas in mice immunized with the non-adjuvanted Ssol protein neutralizing titers fell below the detection limit (Figure 17).

B) Cellular immune response to adjuvanted Ssol protein in C57B1/6 mice

The cell-mediated immune responses of the "Plain", Alum-adjuvanted and 3D-MPL/QS21/ liposome-adjuvanted groups of C57B1/6 mice were investigated as described in Example 3 for the BALB/c mice. However, due to a technical issue with spleen collection, no cellular responses in spleen were available for mice immunized with plain formulations (non-adjuvanted Ssol protein) or mice immunized with Alum-adjuvanted Ssol protein.

CD4+ T cell responses in PBMC

A dose of 2 or 0.2 μg of 3D-MPL/QS21/liposome-adjuvanted Ssol protein induced significantly higher frequencies of CD4+ T cells ($p<0.05$) compared to mice immunized with Alum-adjuvanted Ssol, regardless of dose (Figure 18). With a dose of 0.2 μg Ssol protein, significantly higher ($p<0.05$) CD4+ T cell responses were also induced in mice immunized with 3D-MPL/QS21/liposome-adjuvanted Ssol protein

compared to mice immunized with the non-adjuvanted Ssol protein. Significantly higher ($p<0.05$) CD4+ T cell responses were observed after immunization of mice with 2 or 0.2 μg Ssol protein adjuvanted with 3D-MPL/QS21/liposome compared to mice immunized with 0.02 μg of 3D-MPL/QS21/liposome -adjuvanted Ssol protein. A dose of 0.02 μg Ssol alone or adjuvanted with 3D-MPL/QS21/liposome induced similar frequencies of CD4+ T cells as those induced by immunization with Alum-adjuvanted Ssol or the adjuvant alone.

CD4+ T cell responses in spleen

A trend for higher CD4+ T cell responses was observed after immunization of mice with 2 μg Ssol protein adjuvanted with 3D-MPL/QS21/liposome compared to mice immunized with 0.2 μg of 3D-MPL/QS21/liposome-adjuvanted Ssol protein (Figure 19). Significantly higher ($p<0.05$) CD4+ T cell responses were observed after immunization of mice with 2 μg Ssol protein adjuvanted with 3D-MPL/QS21/ liposome compared to mice immunized with 0.02 μg of 3D-MPL/QS21/liposome-adjuvanted Ssol protein. A dose of 0.02 μg Ssol adjuvanted or not with 3D-MPL/QS21/liposome induced similar level of CD4+ T cell responses as the adjuvant alone.

Cytokine secretion from spleen cells

A trend for higher cytokine production was observed in mice immunized with 2 or 0.2 μg Ssol protein adjuvanted with 3D-MPL/QS21/liposome compared to mice immunized with 0.02 μg Ssol protein adjuvanted with 3D-MPL/QS21/liposome (Figure 20). 2 μg Ssol protein adjuvanted with 3D-MPL/QS21/liposome induced significantly higher ($p<0.05$) IFN-γ than 0.02 μg Ssol protein adjuvanted with 3D-MPL/QS21/liposome.

Summary of results and conclusions for Examples 3 and 4

These data demonstrated that in general the adjuvantation of Ssol protein with 3D-MPL/QS21/liposome adjuvant induced higher levels of anti-SARS-CoV ELISA antibody responses and neutralizing antibody responses in both BALB/c and C57BL/6 mice as compared to immunization with the Ssol protein either in the absence of adjuvant or in the presence of alum. Furthermore, adjuvantation of the Ssol protein

with 3D-MPL/QS21/liposome adjuvant induced higher CD4+ T cell responses and cytokine production in both BALB/c and C57BL/6 mice compared to immunization with Alum-adjuvanted Ssol or the non-adjuvanted Ssol protein. In addition, the Ssol protein with 3D-MPL/QS21/liposome adjuvant provided a Th1-like orientation of the response as indicated by higher production of Th1-type cytokines, lower induction of Th2-type cytokines and an increased production of IgG2a or IgG2b in BALB/c and C57BL/6 mice, respectively.

SEQ ID NO: 1

Amino acid sequence of SARS-CoV #031589 strain S protein

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SGFNTLKPIF KLPLGINITN FRAILTAFSP AQDIWGTSAA AYFVGYLKPT TFMLKYDENG TITDAVDCSQ
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SISITTEVMP VSMAKTSVDC NMYICGDSTE CANLLLQYGS FCTQLNRALS GIAAEQDRNT REVFAQVKQM
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GRLQSLQTYV TQQLIRAAEI RASANLAATK MSECVLGQSK RVDFCGKGYH LMSFPQAAPH GVVFLHVTYV
PSQERNFTTA PAICHEGKAY FPREGVFVFN GTSWFITQRN FFSPQIITTD NTFVSGNCDV VIGIINNTVY
DPLQPELDSF KEELDKYFKN HTSPDVDLGD ISGINASVVN IQKEIDRLNE VAKNLNESLI DLQELGKYEQ
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SEQ ID NO: 2

Ssol amino acid sequence

Amino acids 1-13 correspond to the signal peptide and are cleaved from the mature protein (underlined). Ser-Gly linker and FLAG peptide sequences are in **bold**.

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SGFNTLKPIF KLPLGINITN FRAILTAFSP AQDIWGTSAA AYFVGYLKPT TFMLKYDENG TITDAVDCSQ
NPLAELKCSV KSFEIDKGIY QTSNFRVVPS GDVVRFPNIT NLCPFGEVFN ATKFPSVYAW ERKKISNCVA
DYSVLYNSTF FSTFKCYGVS ATKLNDLCFS NVYADSFVVK GDDVRQIAPG QTGVIADYNY KLPDDFMGCV
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DPLQPELDSF KEELDKYFKN HTSPDVDLGD ISGINASVVN IQKEIDRLNE VAKNLNESLI DLQELGKYEQ
YIK**SGDYKDD DDK**

SEQ ID NO: 3

DNA sequence encoding S protein, inserted within a BamH1-Xho1 cassette, as in pCI-S-WPRE). ATG and TER codons are underlined, extra-sequences (BamH1, Xho1, Kozak sequences are in **bold**).

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CTCGAG

SEQ ID NO: 4

DNA encoding Ssol polypeptide, inserted within a BamH1-Xho1 cassette
- ATG and TER codons are underlined
- extra-sequences (BamH1, Xho1, Kozak sequences are in **bold**)
- Ser-Gly linker sequence is in shading
- FLAG peptide sequence is in box

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TCCACAAATA ATTACTACAG ACAATACATT TGTCTCAGGA AATTGTGATG TCGTTATTGG CATCATTAAC
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AAATATGAGC AATATATTAA ATCCGGAGAT TATAAAGATG ACGACGATAA ATAACTCGAG
```

CLAIMS

1. A vaccine composition comprising:
   (a) an immunogenic SARS coronavirus S (spike) polypeptide, or a fragment or variant thereof; and
   (b) an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.
2. A composition according to claim 1, wherein the S polypeptide consists of or comprises the extracellular domain of the S protein.
3. A composition according to claim 2, wherein the S polypeptide consists of or comprises amino acids 14 to 1193 of the SARS-CoV S protein fused at the C-terminal to the sequence SGDYKDDDDK.
4. A composition according to claim 3, wherein the S polypeptide consists of or comprises the sequence of SEQ ID NO: 2.
5. A composition according to any one of the preceding claims, wherein the S polypeptide is present in an amount of from 1 to 5μg per human dose.
6. A composition according to any one of the preceding claims wherein the lipopolysaccharide is a non-toxic derivative of lipid A.
7. A composition according to claim 6, wherein the lipid A derivative is 3D-MPL.
8. A composition according to any one of the preceding claims, wherein the saponin is an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina.
9. A composition according to claim 8, wherein the saponin is QS21.
10. A composition according to claim 7 or 9 wherein the 3D-MPL and QS21 are present in a ratio of 1:1.
11. A composition according to any one of the preceding claims, wherein the liposome comprises a sterol.
12. The composition according to claim 11 wherein the sterol is cholesterol.
13. The composition according to claim 11 or 12 wherein the ratio of saponin:sterol is from 1:1 to 1:10 w/w.
14. A method of producing a composition according to any one of the preceding claims, the method comprising combining an immunogenic SARS coronavirus

S (spike) polypeptide, or a fragment or variant thereof, with an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.

15. A composition according to any one of claims 1 to 13 for use as a medicament.

16. A composition according to any one of claims 1 to 13 for the prevention or treatment of severe acute respiratory syndrome or other SARS-CoV-related disease.

17. Use of a composition according to any one of claims 1 to 13 for the manufacture of a medicament for the prevention or treatment of severe acute respiratory syndrome or other SARS-CoV-related disease.

18. A method of preventing or treating severe acute respiratory syndrome or other SARS-CoV-related disease, which method comprises administering an effective amount of a vaccine composition according to any one of claims 1 to 13 to an individual in need thereof.

19. A method according to claim 18, wherein the vaccine composition comprises from 1 to 5μg of S polypeptide.

20. A method according to claim 18, wherein the composition is administered in a single-dose vaccination schedule.

21. An immunogenic composition comprising:

(a) an immunogenic SARS coronavirus S (spike) polypeptide, or a fragment or variant thereof; and

(b) an adjuvant comprising a lipopolysaccharide, a saponin and a liposome.

Figure 1

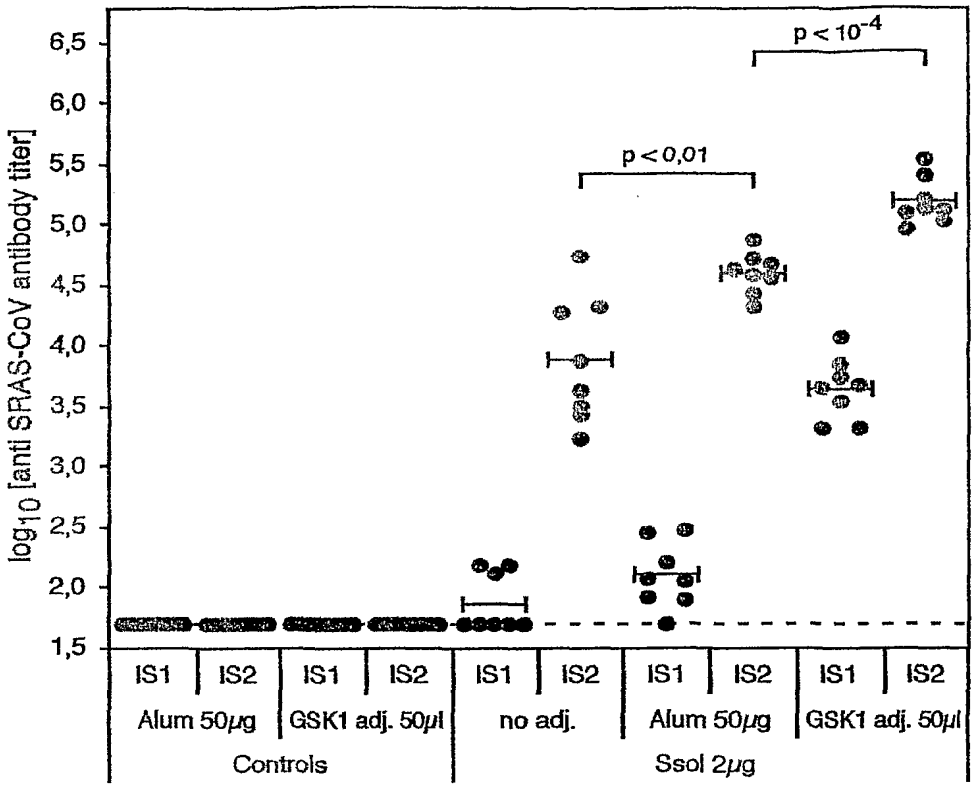


Figure 2

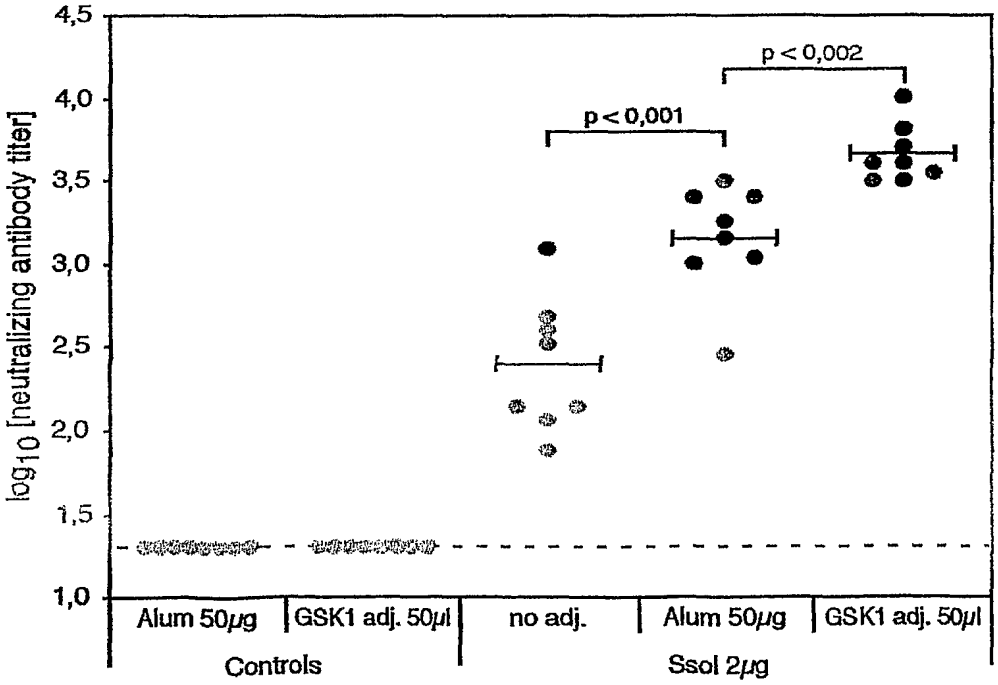
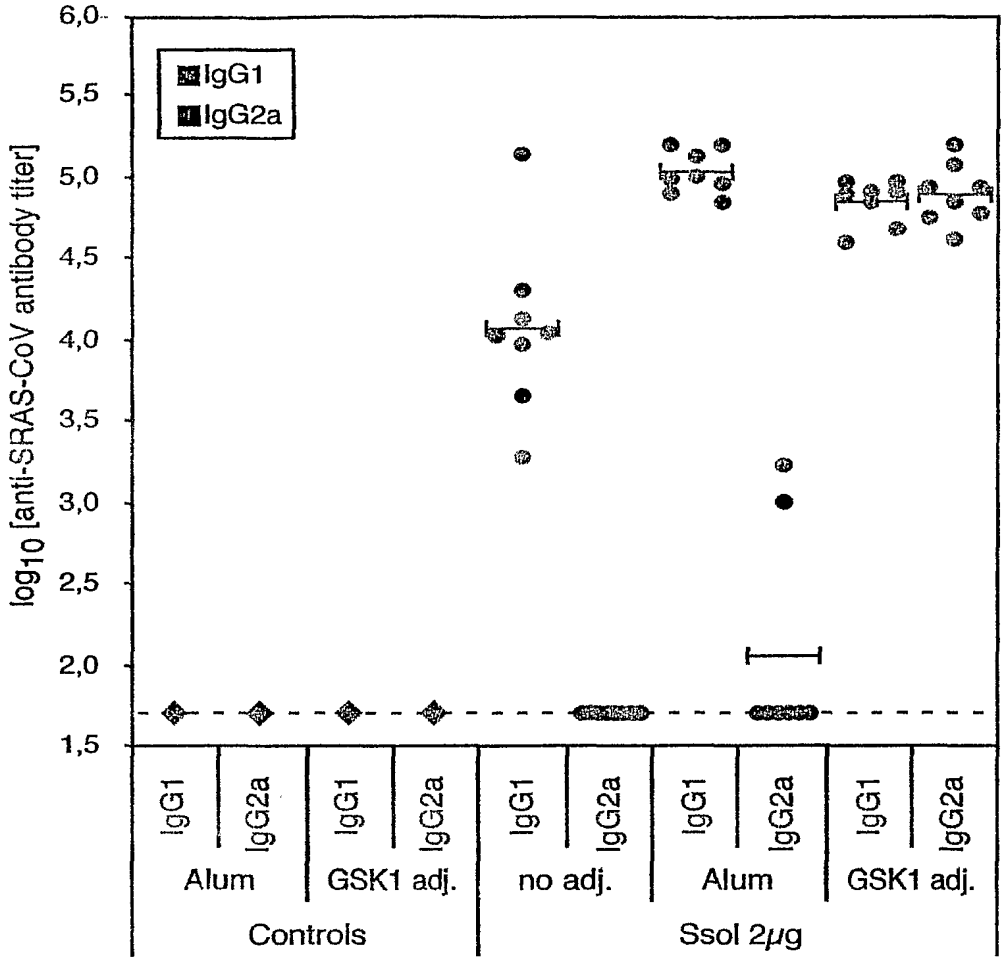


Figure 3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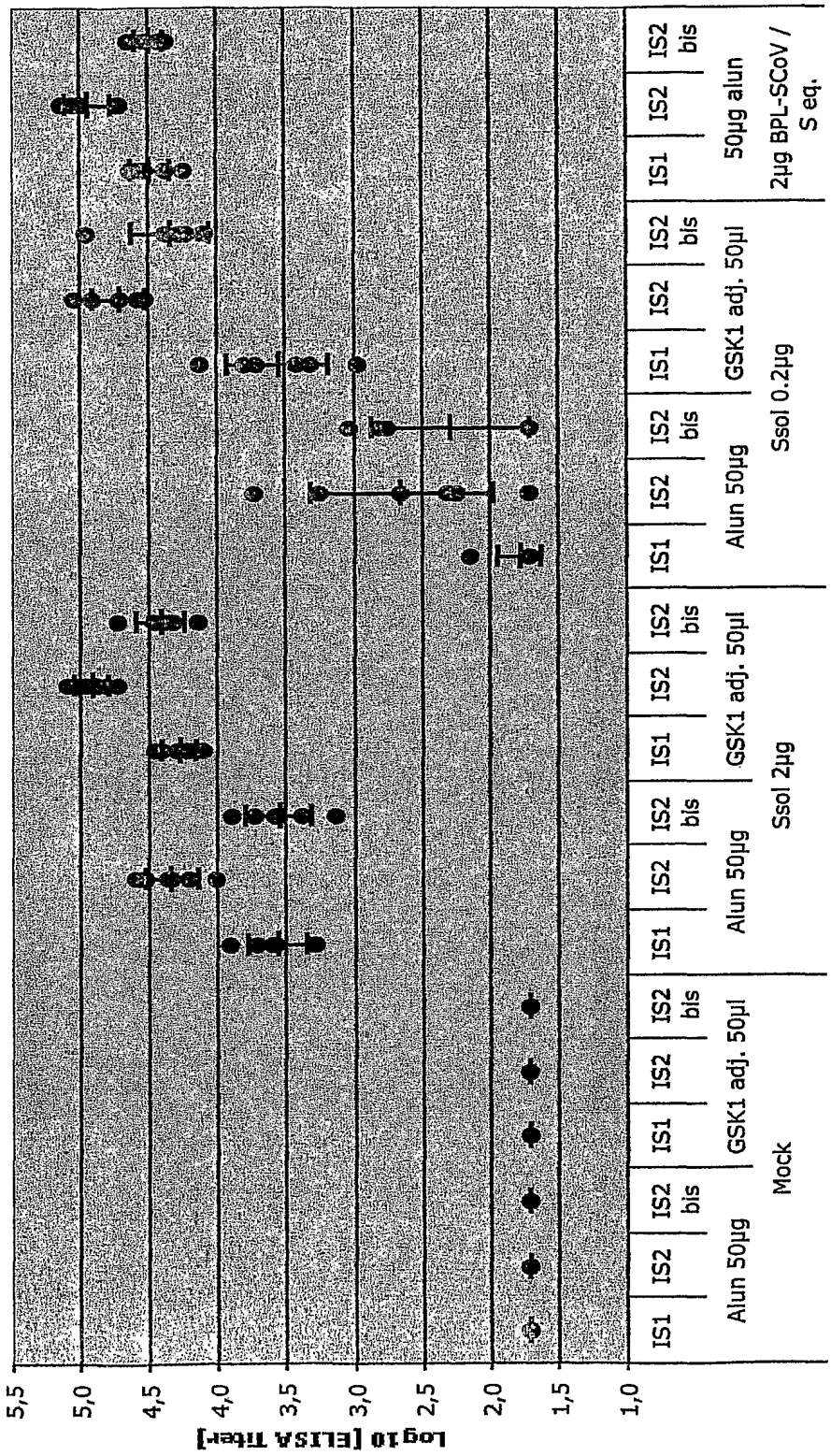
4,5
4,0
3,5
3,0
2,5
2,0
1,5
log10 [anti-SRAS-CoV antibody titer]
IgG1
IgG2a
IgG1
IgG2a
IgG1
IgG2a
IgG1
IgG2a
IgG1
IgG2a
Alum
GSK1 adj.
no adj.
Alum
GSK1 adj.
Controls
Ssol 2μg

Figure 4


Log10 [ELISA Titer]
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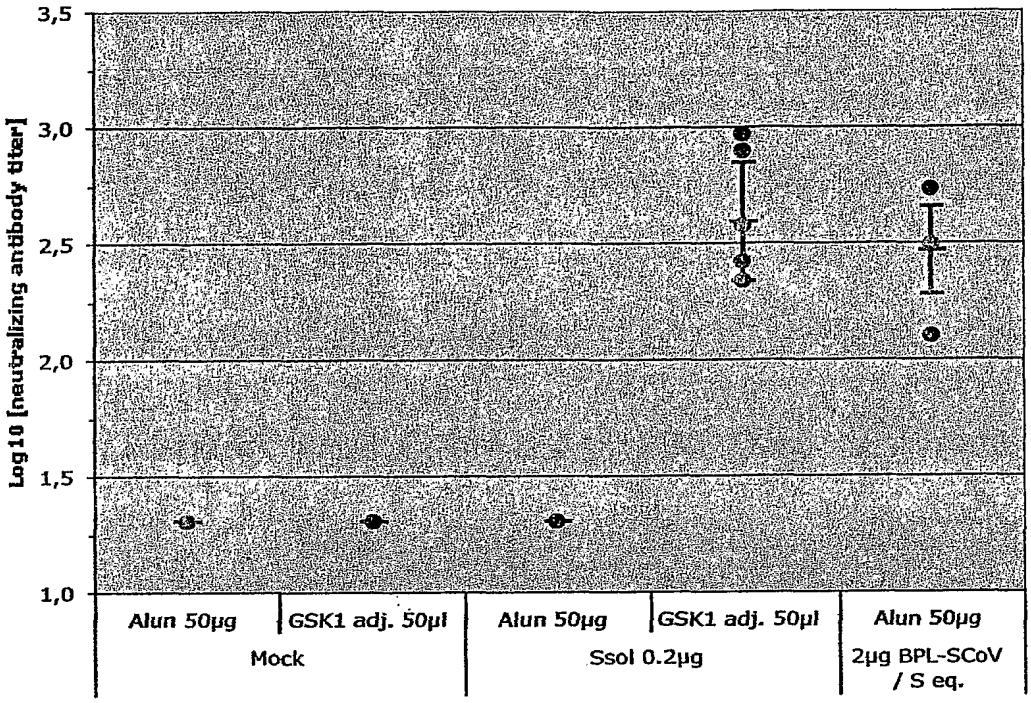
4,0
3,5
3,0
2,5
2,0
1,5
1,0
IS1
IS2
IS2 bis
Alun 50µg
GSK1 adj. 50µl
Mock
Ssol 2µg
Ssol 0.2µg
2µg BPL-SCoV / S eq.
50µg alun

Figure 5



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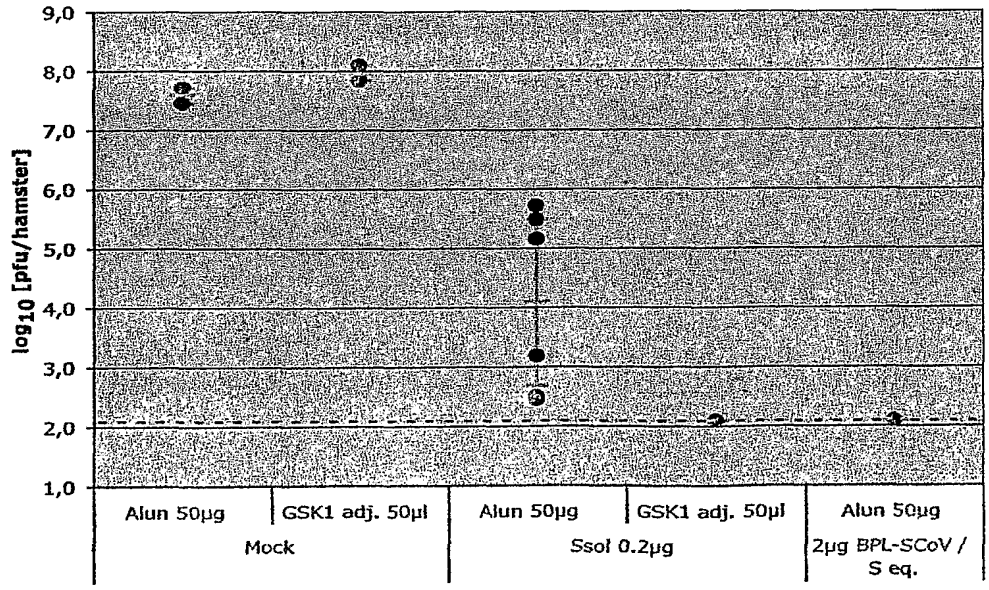

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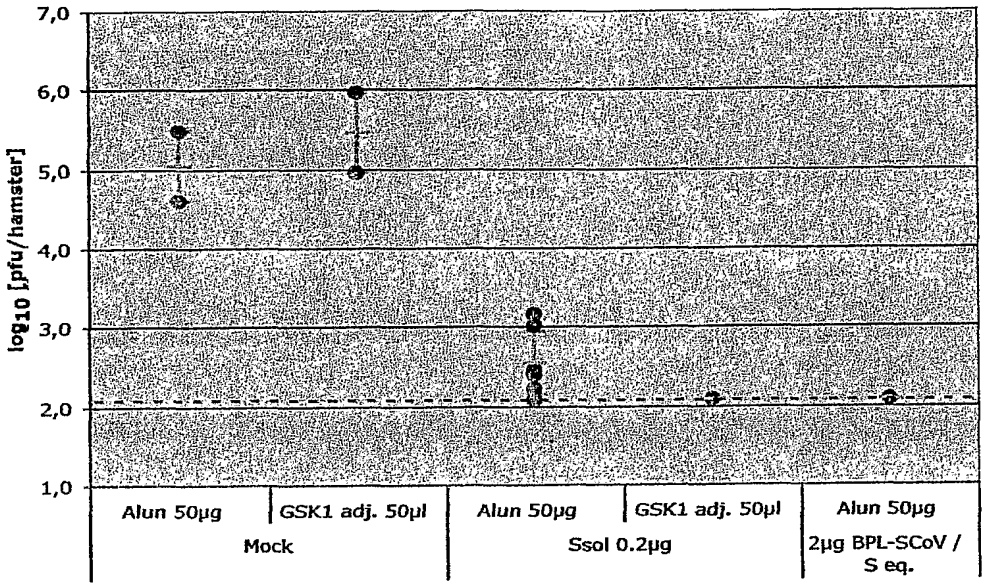

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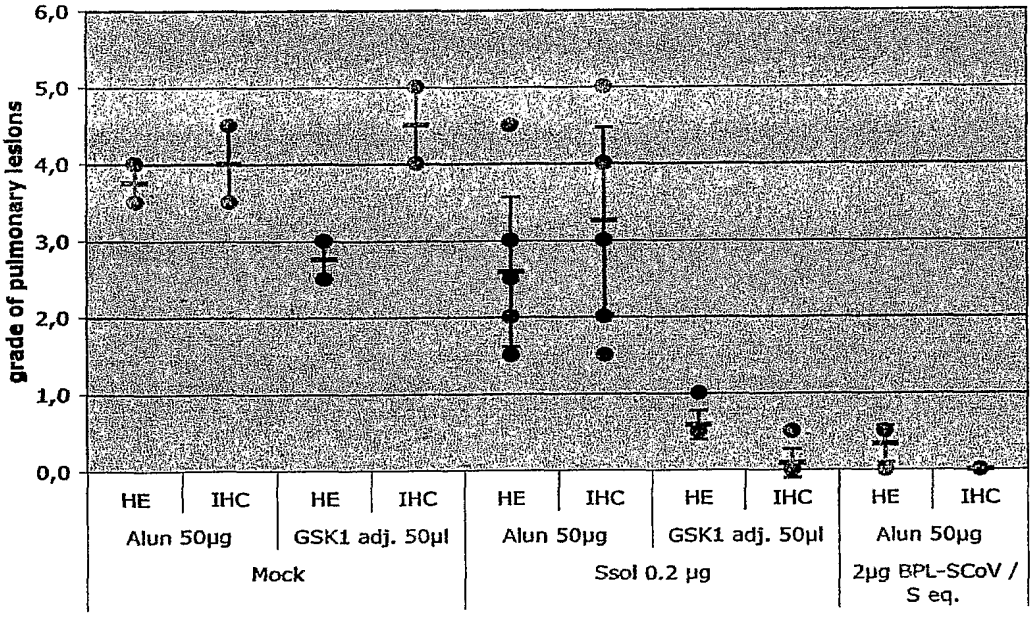

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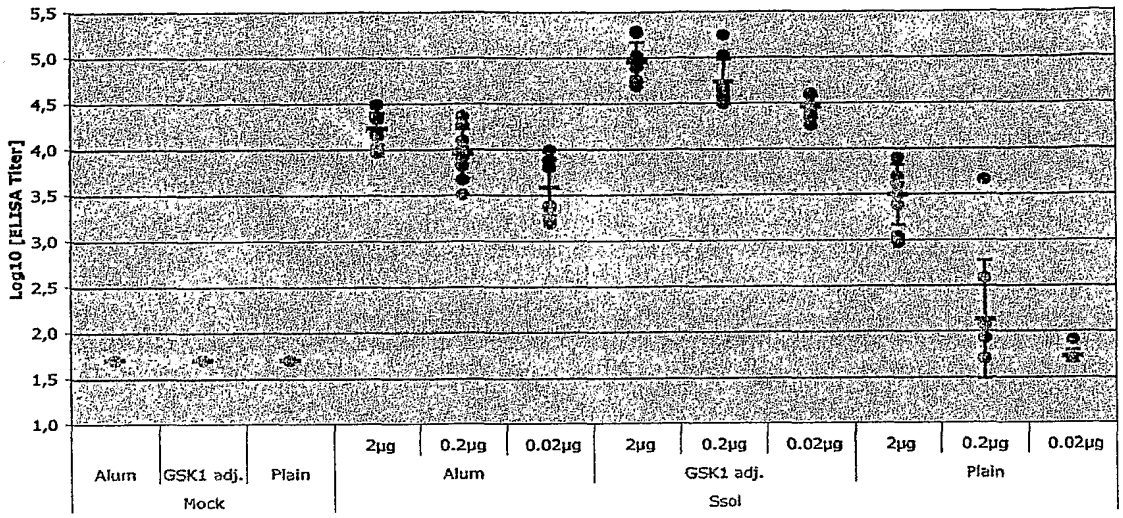

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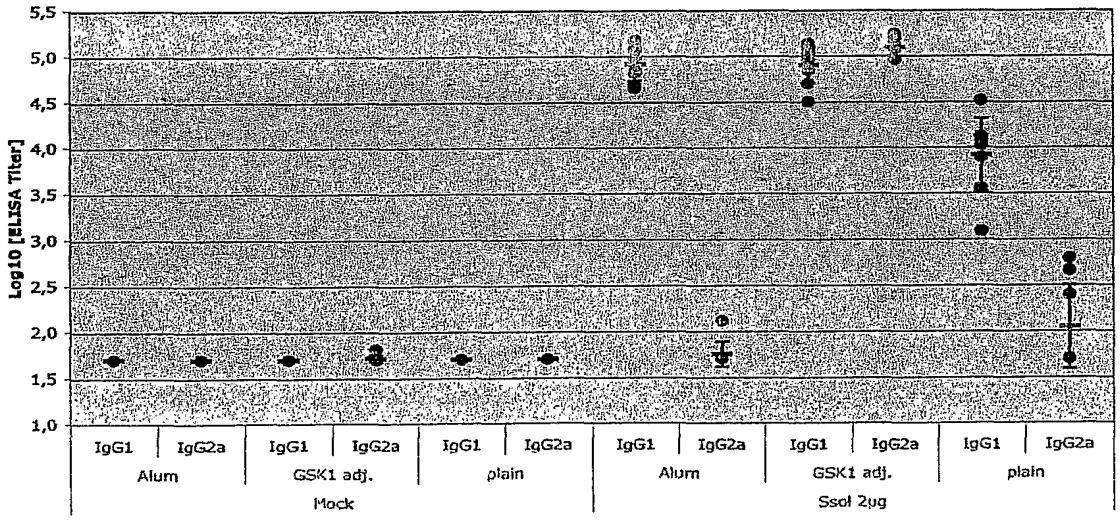

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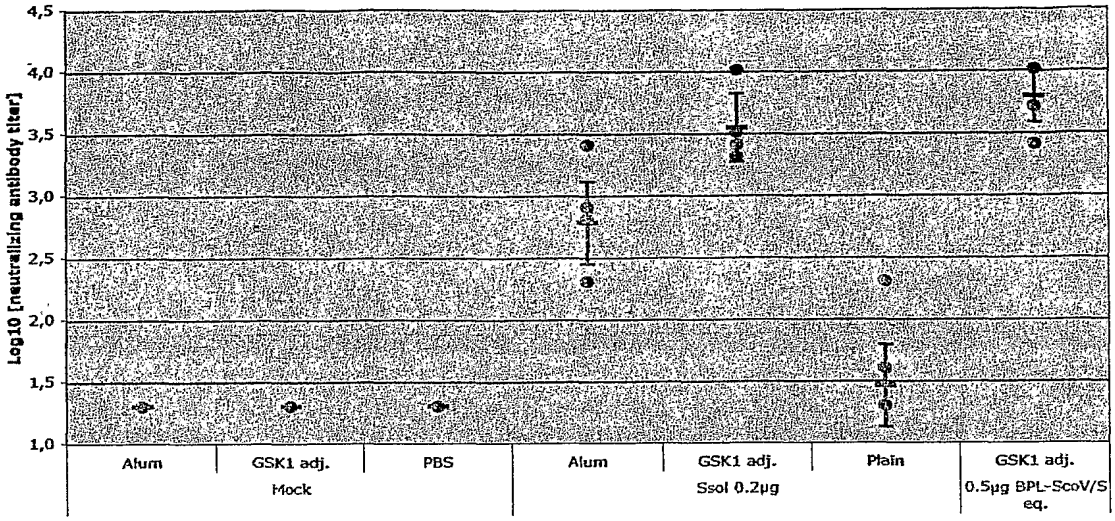

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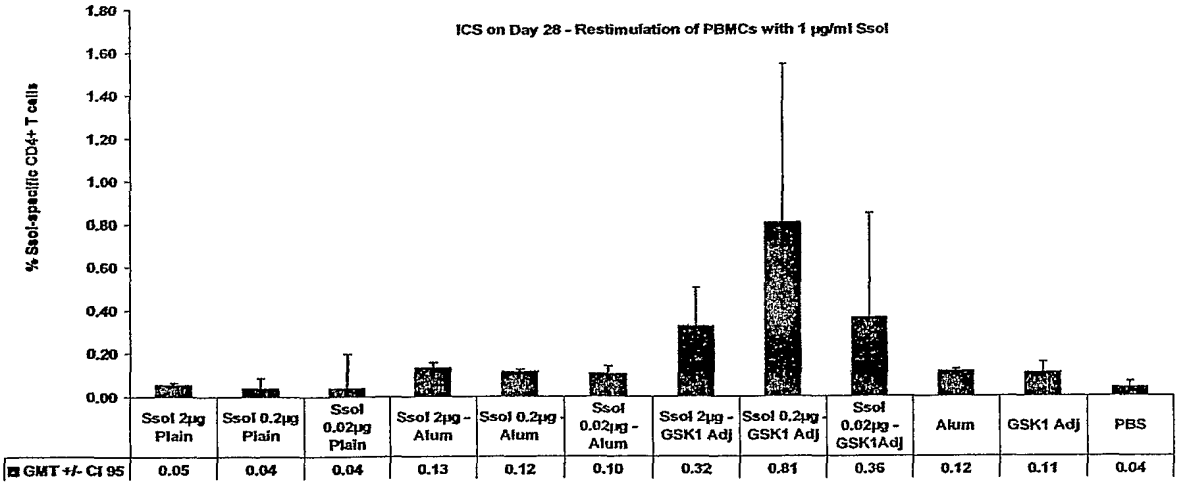

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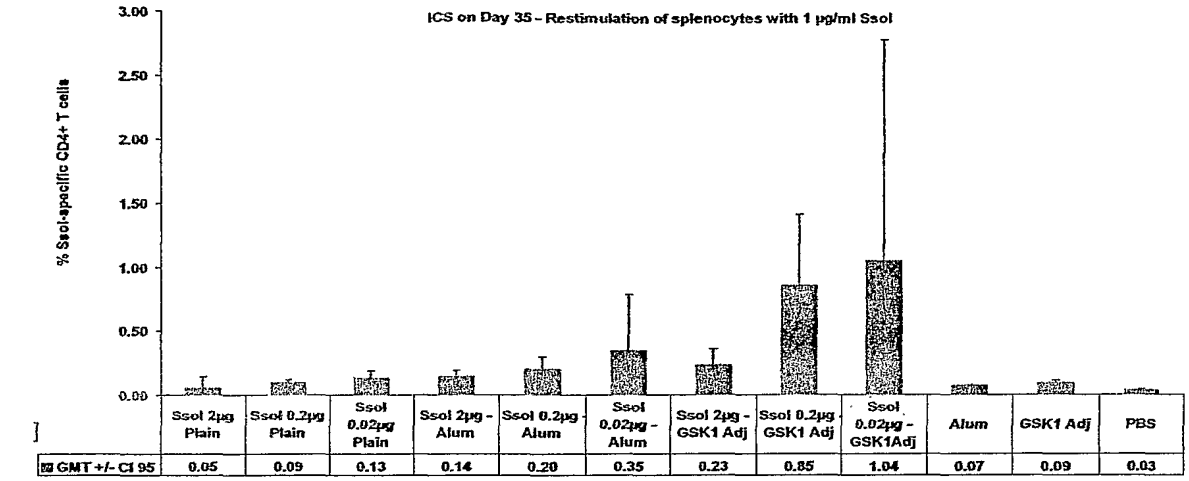
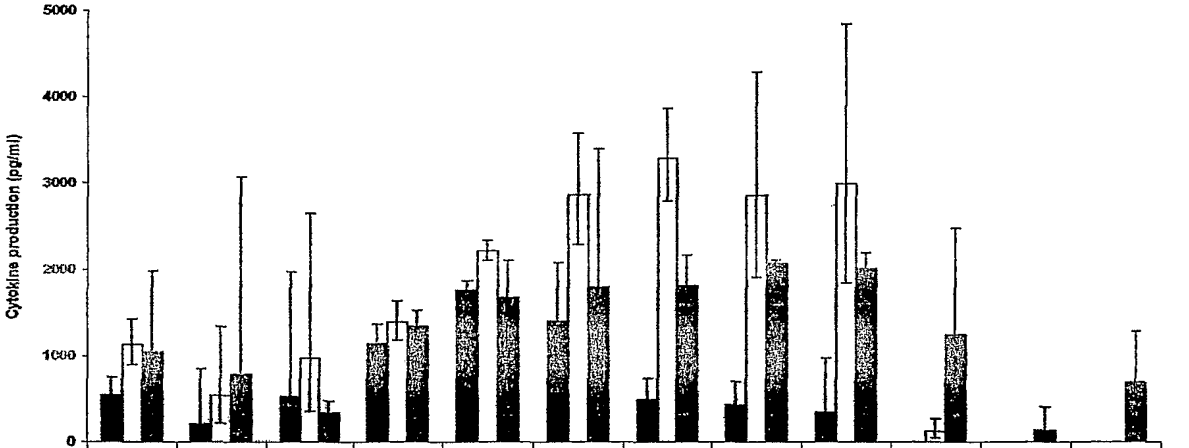
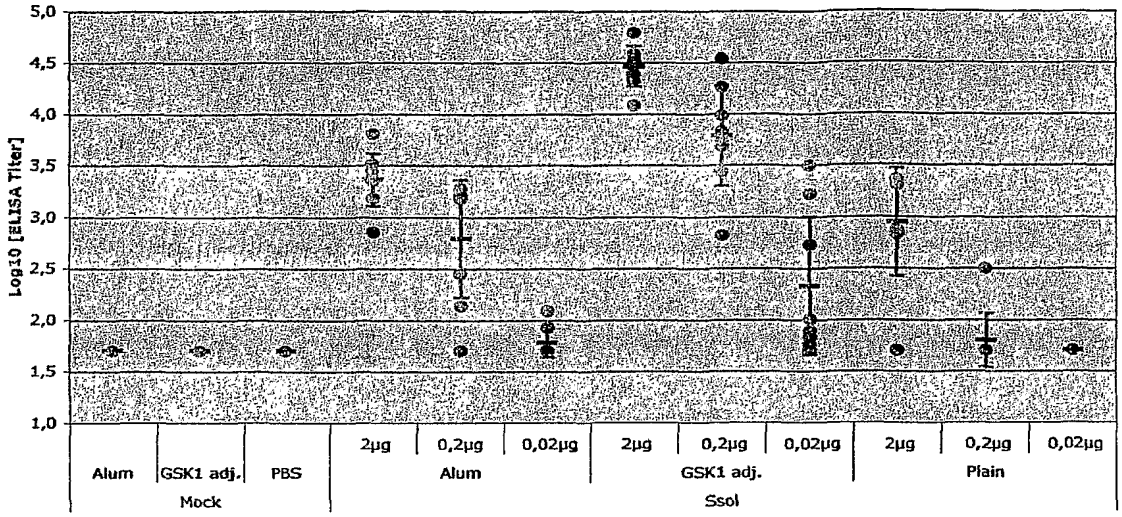


	Ssol 2µg Plain	Ssol 0.2µg Plain	Ssol 0.02µg Plain	Ssol 2µg - Alum	Ssol 0.2µg - Alum	Ssol 0.02µg - Alum	Ssol 2µg - GSK1 Adj	Ssol 0.2µg - GSK1 Adj	Ssol 0.02µg - GSK1Adj	Alum	GSK1 Adj	PBS
GMT +/- CI 95	0.05	0.09	0.13	0.14	0.20	0.35	0.23	0.85	1.04	0.07	0.09	0.03



	Ssol 2µg Plain	Ssol 0.2µg Plain	Ssol 0.02µg Plain	Ssol 2µg Alum	Ssol 0.2µg Alum	Ssol 0.02µg Alum	Ssol 2µg GSK1 Adj	Ssol 0.2µg GSK1 Adj	Ssol 0.02µg GSK1 adj	Alum	GSK1 Adj	PBS
IL-5	538	204	524	1141	1757	1401	488	435	345	5	5	5
IL-13	1133	544	975	1392	2221	2864	3287	2861	2989	119	5	5
IFNg	1045	790	342	1342	1671	1803	1806	2070	2009	1240	141	684

Figure 15



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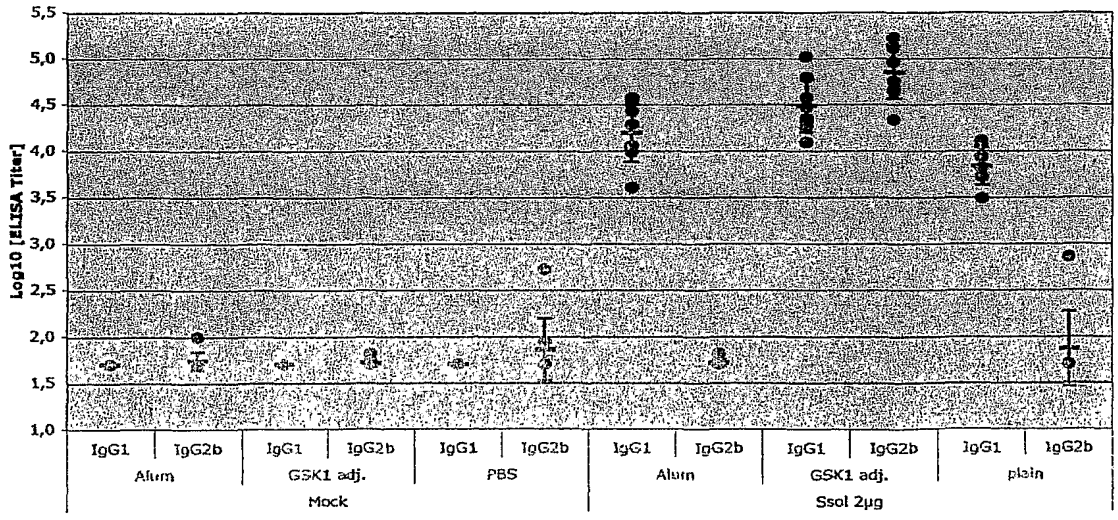

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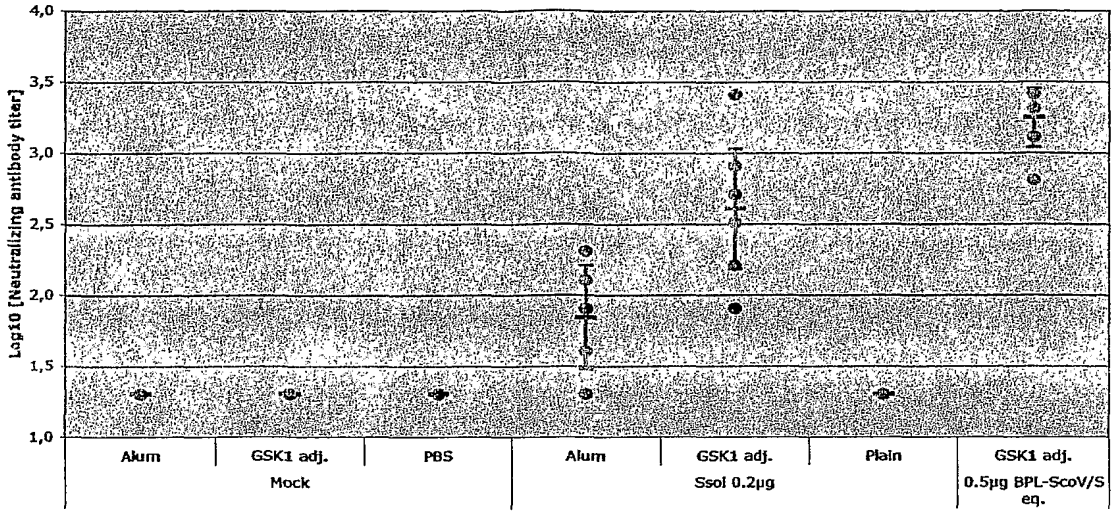

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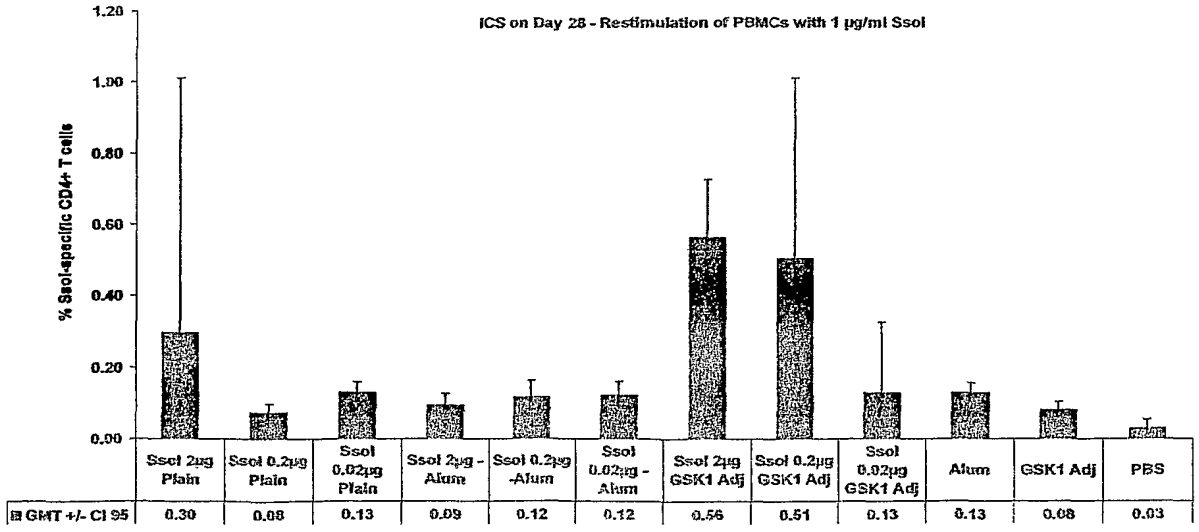

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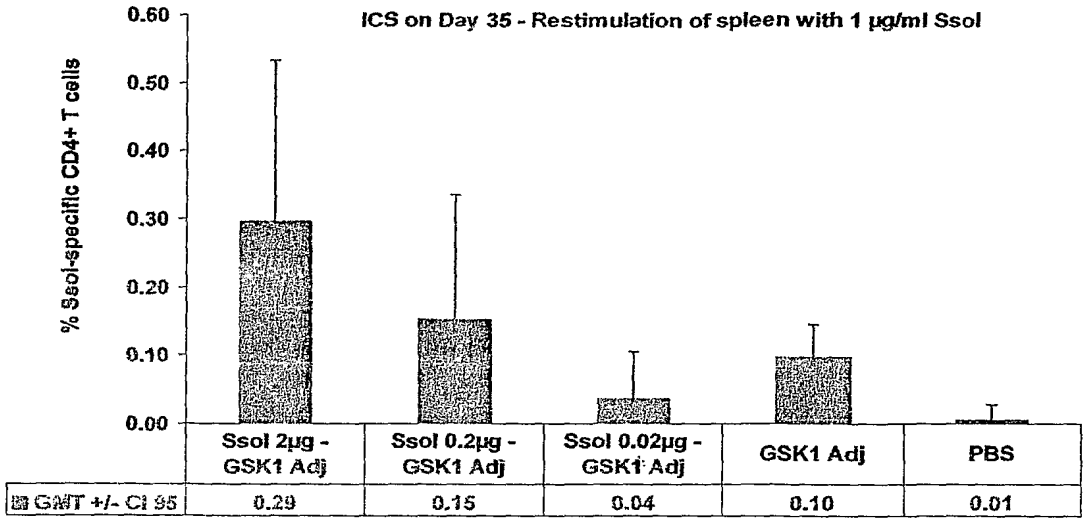
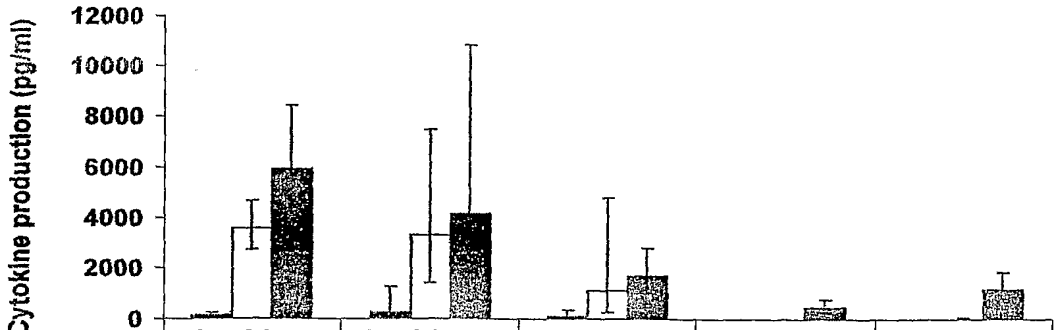


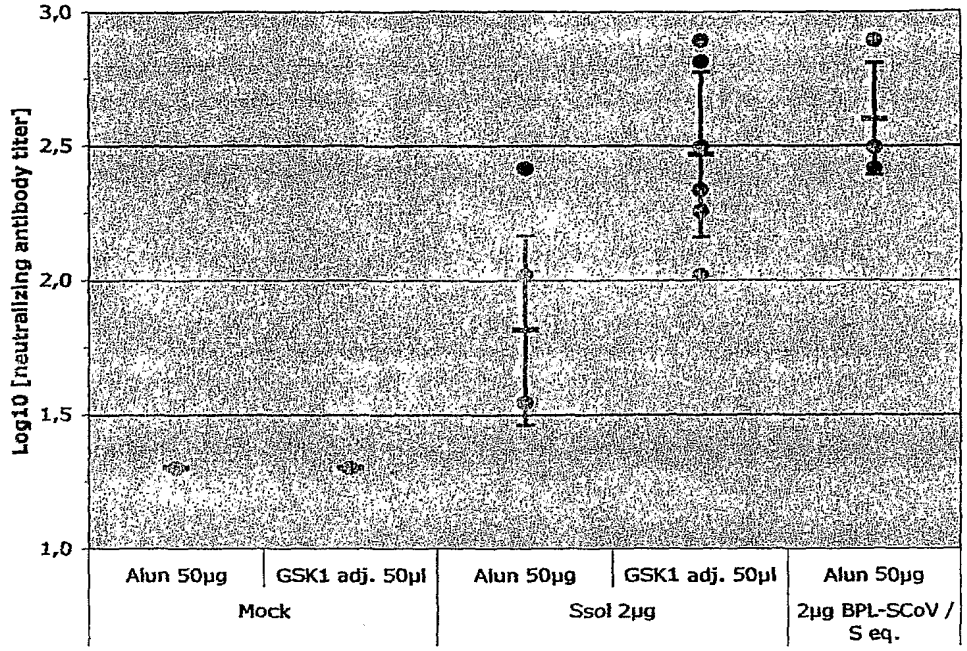
	Ssol 2µg - GSK1 Adj	Ssol 0.2µg - GSK1 Adj	Ssol 0.02µg - GSK1 Adj	GSK1 Adj	PBS
GMT +/- CI 95	0.29	0.15	0.04	0.10	0.01

Figure 20



	Ssol 2µg GSK1 Adj	Ssol 0.2µg GSK1 Adj	Ssol 0.02µg GSK1 Adj	GSK1 Adj	PBS
IL-5	171	258	101	5	5
IL-13	3568	3284	1100	5	11
IFNg	5931	4145	1574	468	1141

Figure 21



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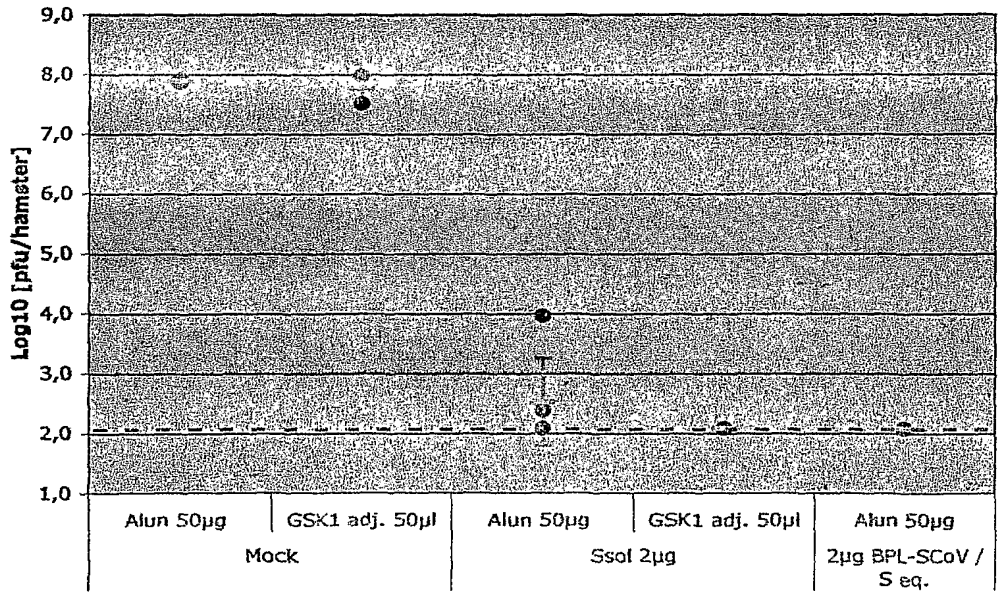

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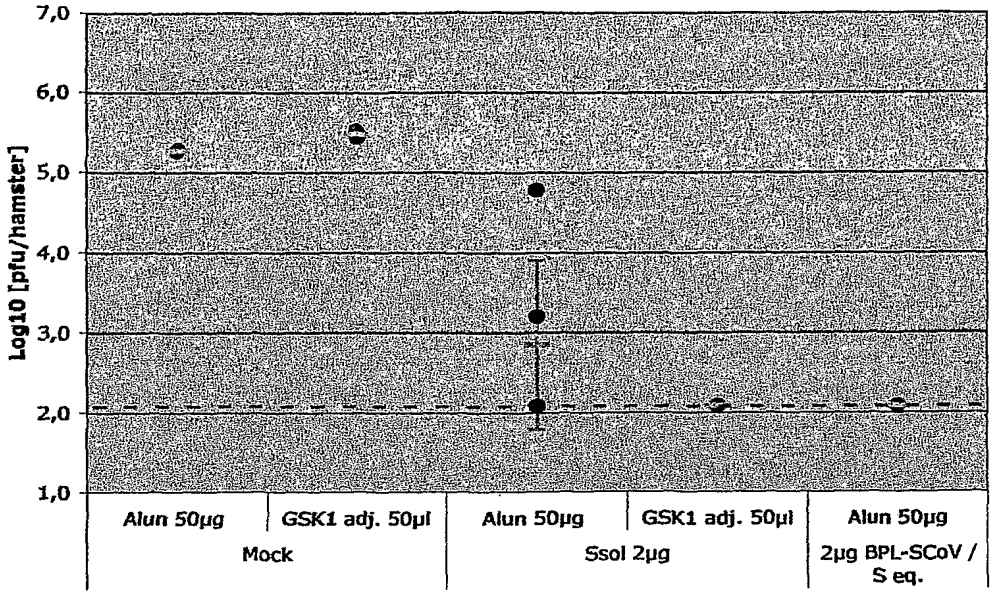

Figure 24